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THE RELATIONSHIP BETWEEN THE LEVEL OF ANTIBIOTIC USE AND RESISTANCE AMONG ENTERIC BACTERIA IN A MULTI-SITE INTEGRATED HUMAN AND SWINE POPULATION

A Thesis

by

KRISTI LYNN CHRISTIAN

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2008

Major Subject: Epidemiology

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Approved by:

Chair of Committee,	H. Morgan Scott
Committee Members,	Virginia R. Fajt
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ABSTRACT

The Relationship between the Level of Antibiotic Use and Resistance among Enteric Bacteria in a Multi-site Integrated Human and Swine Population. (May 2008)

Kristi Lynn Christian, B.S., Texas A&M University

Chair of Advisory Committee: Dr. H.M. Scott

The objective of this longitudinal study was to study the relationship between changes in prevalence of resistant enteric bacteria associated with mean monthly doses (MMD) of various antibiotics used in each of two host species. From January 2004 – January 2007, monthly composite swine fecal samples and human wastewater samples representing various production and occupational cohorts, respectively, were collected from 19 geographically unique locations in east- and south-central Texas. Bacterial isolates cultivated on CHROMagar-E.coli™ and Difco™ mEnterococcus (ME) were tested for susceptibility to multiple antibiotics by microbroth dilution using the Sensititre™ system. The relationship between the prevalence of resistant bacteria, sampling period, and antibiotic use within each host species was assessed in a generalized linear model adjusted for the dependence of responses within location using a binomial distribution and logit link function in STATA® ver. 9.2.

For the swine *E. coli* isolates, the relationship between tetracycline resistance and level of chlortetracycline (CTC) use in swine illustrated a dose-response relationship,

with odds ratios (OR) of 1.20 and 1.81 ($P < 0.05$) for second- and third-level categories of MMD relative to baseline (zero-use) respectively. When considered by swine production groups, intake boar isolates had an elevated relative odds of resistance to tetracycline (OR = 1.51, $P < 0.05$), and the nursery units had an elevated odds (OR = 2.61, $P < 0.05$) of exhibiting resistance to ceftiofur, relative to pigs housed in the farrowing barns. Regarding swine *Enterococci* isolates, those swine from locations that utilized tylosin had an elevated OR of 3.54 ($P < 0.05$) of exhibiting resistance to tylosin, relative to those locations that used no tylosin. At this juncture, an apparent occupational risk of harboring tetracycline-resistant *E. coli*, and the apparent sparing effect (*Enterococcus* spp.) associated with exposure to swine production, remain unexplained.

This study demonstrated that the prevalences of tetracycline- and tylosin-resistant enteric bacteria swine were dependent on CTC and tylosin use in feed, respectively. Swine production group-effects on the prevalence of tetracycline, ceftiofur, and erythromycin resistance were also important. This study provides a better understanding of the relationships between antibiotic prescribing practices at the ecologic level and the relative odds of carriage of resistant bacteria within two host species in a vertically integrated agri-food system.

DEDICATION

I would like to dedicate this thesis to my family. It is through their unwavering love and support that I have accomplished my goals. They have kept me grounded in the faith and reminded me of all the many blessings God has given.

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First, I would like to thank my committee chair, Dr. H. Morgan Scott, for challenging me to think "outside of the box". As a result of his teaching, I have acquired skills and knowledge that will only enhance my future career as a physician. I would like to thank Dr. Virginia Fajt for her prompt replies to all of my questions throughout the course of this project; it was greatly appreciated. Also, I would like to thank Dr. Roger Harvey for his excellent editorial skills and the many chocolate bars I ate during our meetings. Finally, I would like to thank Dr. Bruce Lawhorn for the many hours he spent collecting samples and patiently answering all of my questions about pigs and pig production.

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CHAPTER I

INTRODUCTION

Antibiotics[1] were first identified and introduced into use in the early twentieth century. Antibiotics are natural or synthetic compounds that either kill or inhibit growth of bacterial microbes that can cause disease in humans or animals (Centers for Disease Control and Prevention, 2006). Antibiotic resistance is the ability of a bacteria to grow in the presence of an antibiotic that would normally kill or inhibit its growth. The net result may be to reduce the effectiveness of drugs, or other agents to cure or prevent infections. The first documented reports of clinically relevant antimicrobial resistance to sulfonamides appeared in 1939, to penicillin in 1942, and to streptomycin in 1946 (MacLean et al., 1939; Fleming, 1942; Klein et al., 1946). Development of resistance to antibiotics has become one of the most pressing international public health concerns (Centers for Disease Control and Prevention, 2006). Antibiotic resistance is a leading concern for agencies such as the Centers for Disease Control and Prevention (CDC), the United States Department of Agriculture (USDA), as well as the Food and Drug Administration (FDA) which regulates the approval, marketing, and use of antibiotics in both human and animal populations in the United States. A recent and well-known

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This thesis follows the style of Preventive Veterinary Medicine.

[1] An antibiotic is a drug used to kill or inhibit bacterial growth and may be considered one of the antimicrobial agents. Antimicrobial is a general term for drugs, chemicals, or other substances that kill or slow the growth of microbes.

example of the association of antibiotic use in farm animals on resistance levels in the animal and human populations is the temporal link between avoparcin approval in the poultry industry in Europe, and the resulting increase of vancomycin-resistant *Enterococci* (VRE) in the European poultry and human populations (Wegener et al., 1999). This finding, among others, resulted in the animal-health formulation of the product being withdrawn from the market in Europe (Wegener et al., 1999). It is important to note that avoparcin was never approved for use in North America, and VRE remain exceedingly rare in the North American livestock and poultry populations (Poole et al., 2005). Recent reports have prompted the development of programs to educate both the public and health-care providers (human and animal) on prudent use guidelines for the treatment for infections with antibiotics (McDonald et al., 1997; van den Bogaard et al., 2001). Some authors contend that inappropriate and repeated use of antibiotics in human medicine is primarily responsible for the rise in resistant bacteria (Centers for Disease Control and Prevention, 2005b). The CDC have estimated that in the United States nearly 90,000 people die each year from infectious diseases with 70% of these caused by bacteria that are resistant to at least one commonly used drug (Centers for Disease Control and Prevention, 2005a).

The use of antibiotics for nontherapeutic purposes in healthy animals in order to promote growth or prevent disease is heavily debated, as it is believed that the agricultural use of these drugs has resulted in pressures selective for the emergence of resistant bacteria. These bacteria or their genetic material encoding for resistance may

then be transferred to humans through the food supply, by direct contact with animals, or via waste runoff from animal production facilities. In the year 2000, the World Health Organization (WHO) recommended the ban of antibiotics in food animals for nontherapeutic purposes, unless a risk-based evaluation proved their safety (WHO, 2000). Although antibiotics have been approved for growth promotion and improved feed efficiency in the U.S., there is no centralized reporting system useful for relating the quantity of antibiotic use in food animals and the level of resistance in human food consumers. International organizations such as the Food and Agriculture Organization (FAO) of the United Nations, the Office International des Épizooties (OIE: World Organization for Animal Health), and the WHO have recognized this problem and recommend that each country establish a monitoring system (Nicholls et al., 2001; WHO, 2001). Systems that may be used by agencies to monitor levels of resistance alongside antibiotic use may be useful in answering questions concerning the quantities of antibiotics used in the food animal industry and their effect on resistance levels.

Public concern about the potential effects of antibiotic use in farm animals on the levels of resistance in enteric bacteria colonizing the human population – particularly pathogenic enteric bacteria - has led to increased research in this area. For example, in the United States, fluoroquinolone-resistant *Campylobacter* spp. have been linked to flouroquinolones used in treatment of respiratory diseases in poultry. The strains found in poultry were very similar to those found in humans leading the researchers to hypothesize that fluoroquinolone use in poultry was a major contributing factor

(Anderson et al., 2003). Another research effort to compare enteric bacterial strains of animal and human origins found identical bla_{CMY-2} genes in resistant *E. coli* and *Salmonella* spp. isolates from cattle, swine, and human samples (Winokur et al., 2001). Although identical genes in resistant bacteria have been found, it has not been definitively proven that transmission (and subsequent colonization) between host species can (or have) taken place as a direct result of antibiotic use in food animals.

In response to multiple study findings and evidence regarding the relationship between antibiotic use in food animals and the rise in resistance of enteric bacteria of both human and animal origins, the National Antimicrobial Resistance Monitoring System (NARMS) for enteric bacteria was created in 1996 in order to better monitor the levels of resistance in enteric bacteria (Centers for Disease Control and Prevention, 2007). NARMS is a collaboration of the CDC, the FDA (Center for Veterinary Medicine) and the USDA (both the Food Safety and Inspection Service (FSIS) and the Agricultural Research Service (ARS)). NARMS is responsible for testing and recording the resistance results for non-Typhi *Salmonella* isolates, *Salmonella* Typhi, *Shigella*, and *E. coli* O157:H7 isolates received from participating public health departments and a variety of veterinary sources. The information gathered is used to evaluate trends in resistance in order to better guide the development of regulatory policies regarding the use of drugs in food producing animals (Centers for Disease Control and Prevention, 2004).

To aid in evaluating the trends in resistance levels within and among human and food-animal populations, longitudinal studies may be employed. It is presently difficult to evaluate the association of antibiotic use in the food animal populations with levels of resistance in the human population due to high human mobility, multiple and disparate sources of food (specifically, meat, milk and poultry products), and lack of antibiotic usage data. Since bacterial resistance may also arise due to physicians' over-prescribing of antibiotics and individual patient non-compliance or antibiotic abuse, it is problematic to assume that antibiotic use in food animals is the major contributing factor to resistance levels. Scientific studies need to be conducted in order to better assess this relationship, beginning with more carefully defined and closed populations, with known sources of occupational and food exposure to enteric bacteria, and with more accurate antibiotic use data from both the animal and human populations.

Objectives

This study is one component of a large-scale 3-year multi-site longitudinal project and is specifically designed to investigate the temporal and spatial relations between the levels of antibiotic-resistant phenotypes and genotypes of generic and pathogenic enteric bacteria (specifically *Escherichia coli* (*E. coli),* various *Salmonella enterica* serovars, and *Enterococcus* spp.), and concurrent antibiotic use in both swine and human species. The specific objectives of this longitudinal study are to: 1) examine the antibiotic resistance phenotypes of certain commensal enteric bacteria (specifically *E. coli* and *Enterococcus* spp.) isolated from aggregated monthly wastewater samples

from humans and composite fecal matter from swine, and 2) to study the relationship between the prevalence proportion of these resistant bacteria and the amount and type of antibiotics used in both host species (i.e., varying by host, occupational or production cohort, month or season, and geographical location). The benefits of our research pertain to a better understanding of the relationships between antibiotic prescribing practices at the ecologic level (in both humans and swine) and the relative odds of carriage of resistant bacteria within and among host species in a vertically integrated agri-food system.

CHAPTER II

LITERATURE REVIEW

Introduction to Antibiotics

Before the introduction of antibiotics, human and veterinary medical practitioners had limited options when dealing with bacterial infections. The beginnings of clinical application of antibiotics in the 1930's and 1940's ushered in a new era of progress in medicine, but along with this progress a new problem of resistance arose (Harris and Kahn, 1940; Lowell et al., 1940; McKee and Rake, 1942). Antibiotics were becoming less affective in killing of inhibiting bacterial growth. Over the next 60 years, the number of resistant bacterial strains steadily increased, recently culminating in the WHO listing antibiotic resistance (AR) as a leading world health issue (Centers for Disease Control and Prevention, 2006).

Antibiotics work by interfering with various bacterial functions, such as cell wall synthesis, protein synthesis, and nucleic acid synthesis (Bonafede and Rice, 1997; Witte, 1998; Tenover, 2006). Bacteria may become resistant through spontaneous mutation or else acquire resistance through a number of sexual- and asexual-reproductive and other genetic mechanisms. After acquiring resistance, the bacteria may pass from human to human, animal to animal, or from food animals to humans through the food chain (Anderson et al., 2003).

Selection pressures, such as those provided by antibiotic use, can improve the probability of survival and propagation of resistant bacterial strains. When bacteria are initially introduced to antibiotics, the susceptible bacteria die resulting in a population consisting only of those bacteria that express resistance. In a Swedish study, scientists conducted a cohort study in order to investigate the effects of antibiotic treatment over a 1 year period on commensal flora in humans (Gustafsson et al., 2003). A significant increase ($P = 0.0001$) in rifampicin resistance was observed for *E. coli* in patients with antibiotic treatment over a one year period compared to those patients with no antibiotic treatment. Although this study does support the hypothesis that bacteria with a high mutation rate are heavily influenced by exposure to antibiotics, study limitations included that many of the patients exhibited compromised immune systems. Bacterial mutation and proliferation may have been reduced in patients with non-compromised immune systems. Selection pressures can also include environmental conditions. A study conducted with Belgian fattening pigs found a significant association of AR with pen hygiene (Dewulf et al., 2007). This finding supports the selection pressure hypothesis. According to this theory, susceptible bacteria could have been more readily removed during cleaning, allowing the resistant bacteria to multiply and become the dominant strains.

Mutation is not the only mechanism through which bacteria acquire resistance to antibiotics; they may also receive and transfer resistance genes to other bacteria; both within and between species (Tenover, 2006). One example of a bacterium that is able to

both receive and transfer genetic resistance is *Escherichia coli*. As coded through these genetic elements, bacteria may generate enzymes that destroy the antibacterial agent, develop pumps that eject the agent, change the cell wall so there are no binding sites, or mutate so the agents are unable to reach the target site (Tenover, 2006).

Aside from the selective pressure applied by antibiotic use, one study also found an association between population density and antibiotic resistance (Bruinsma et al., 2003). The researchers considered three different cities and measured the defined daily dosages (DDD) per 1,000 inhabitants by square mile. Adjusted for DDD, the highest prevalence of antibiotic resistance was found in the city with the highest density. This study had several potential confounding limitations including; city location (located on different continents), antibiotic recording systems (recorded in different years), low response rates, and other forms of selection bias. Further studies need to be conducted to provide better insight into the population density theory. Unique units within the same geographic area, but with different population densities need to be considered instead. In addition, a consistent system of antibiotic dispensing records should be used to give a more accurate account of DDD. If the assumption is that population density influences antibiotic resistance, then the most populated units should have the highest level of resistance, all other factors (i.e., DDD) being held constant.

Escherichia coli

Escherichia coli (*E. coli*) are facultative anaerobes and are part of the normal intestinal flora in humans and animals (Schroeder et al., 2002a). The intestinal flora play

an important role in the immune response, food digestion, and prevention of the colonization of the intestinal tract by pathogens (Gustafsson et al., 2003). The *E. coli* bacterium belongs to the Enterobacteriaceae family and is ubiquitously found in the feces of healthy humans, swine, and other domestic and wild mammals and birds. Some pathogenic strains of *E. coli* are responsible for several infectious diseases in humans (e.g. urinary tract infections, neonatal meningitis, septicemia, and surgical site infections) (Schroeder et al., 2002a). Since commensal (non-pathogenic) *E. coli* are highly prevalent, and are easily isolated in the feces of healthy humans and animals, they are commonly used as indicator bacteria (Scott et al., 2005). The differences in prevalence of resistance to one or more antimicrobials (within and among host species) may be used to assess the short- and long-term effects of variable selective pressures applied in those same host communities (O'Brien, 2002; Schroeder et al., 2002b).

The spread of resistant *E. coli* between host-species suggests that bacteria harboring the resistance genes may spread from food-producing animals to humans. *Escherichia coli* exposure of humans may occur via fecal contamination of carcass meat during food animal slaughter, or via direct contact with the animals (Piddock, 1996; Van Den Bogaard and Stobberingh, 2000; Scott et al., 2005; Dewulf et al., 2007). Studies to research this link have been conducted in Spain and Taiwan where *E. coli* strains with reduced quinolone susceptibility were found in both humans and poultry. Since quinolones are not used in treatment for children, the reduced susceptibility found in these studies raised concerns of the transfer of resistance through the food chain

(O'Brien, 2002). Other studies have found that the use of certain drugs such as penicillins, sulphonamides, cephalosporins, and tetracyclines greatly influences the relative proportions of AR *E. coli* in feces (Huycke et al., 1998; Bonten et al., 2001).

Enterococcus spp.

Enterococci are gram-positive facultative anaerobes and are commonly found in the intestinal tracts of healthy humans and animals (Murray, 1990). *Enterococci* may cause endocarditis, bacterimia, urinary tract infections, neonatal infections, and other nosocomial infections (Murray, 1990). The *Enterococcus* genus contains many strains that are almost completely resistant to antibiotics (DeLisle and Perl, 2003).

Because enterococci are often resistant to many antibiotics, one treatment reserved almost exclusively for enterococcal and staphylococcal infections is the antibiotic vancomycin, often used in combination with other antibiotics. Unfortunately, in the late 1980's, reports of vancomycin resistance began to surface and the health care community has since had to reassess enterococcal therapy. Newer and more powerful drugs must now be employed in cases where vancomycin resistance is present. The first reports of vancomycin-resistant enterococci (VRE) in the United States were in 1988, when researchers found resistant strains in infected hospital patients and one asymptomatic hospital patient (Uttley et al., 1988). By 1997, more than 15% of nosocomial infections in U.S. hospitals were caused by VRE (Wegener et al., 1999). Although the spread of VRE in Europe is believed to be a result of the use of avoparcin in food-animal populations, studies have indicated that in the United States VRE may be

transferred from the hospital environment to the human community (Wegener et al., 1999; DeLisle and Perl, 2003). Since the European ban of avoparcin in 1997, studies have found a high prevalence of VRE in pig feces and slurry (Manero et al., 2006). This led to the hypothesis that the past use of tylosin as a feed additive may have selected for vancomycin resistance genes retarding the rate of decline of VRE prevalence despite the ban.

Non-therapeutic and Therapeutic Antibiotic Usage in Food Animals

In the early 1970's an important study found resistant *E. coli* not only in the feces of hospital patients, but also in the feces of healthy humans, animal carcasses in the slaughter plant, and hospital food (Cooke et al., 1971). These findings introduced the idea of resistance transfer from food animals to humans. Antibiotics have been used in the food-animal industries for roughly 50 years and the effects of this use continue to be examined.

Some scientists hypothesize that those antibiotics used in food animals that have a human analog may be responsible for the increasing AR found in human populations (Anderson et al., 2003). Resistant bacteria may pass to agricultural workers through direct contact with animals or their feces, or to the consumer through the food chain. In 1983, nourseothricin was introduced as a growth promoter in the German swine industry. Before the introduction of nourseothricin, the prevalence of resistant *E. coli* in human and animals was extremely low. By 1985 nourseothricin resistance was found in pigs, and by 1990 the resistance had been found in pig farmers, their families, and in

hospital patients (Witte, 1998). In 2005, a study conducted at slaughter plants in Great Britain found multiple cephalosporin-resistant *E. coli* strains in cattle feces, and recently, extended-spectrum cephalosporin resistance in humans also has been found (Winokur et al., 2001; Schroeder et al., 2002a; Batchelor et al., 2005). Ceftiofur is the only 3rd generation cephalosporin approved for use in food animals (in 1988, the FDA approved ceftiofur for use in cattle and in 1992 it was approved for use in swine in the United States) and these data suggest the possible transfer of resistant *E. coli* from food animals to humans. A high rate of resistance to other antibiotics such as gentamicin, tobramycin, streptomycin, tetracycline, trimethoprim-sulfamethoxazole, and chloramphenicol was also found.

As mentioned earlier, another European example of resistant bacterial transfer from animals to humans is the VRE. Genetically similar strains were isolated from animals and humans, thus suggesting that resistant bacteria may be transferred across species (DeLisle and Perl, 2003). In the south of The Netherlands, highly resistant VRE were isolated from turkeys, turkey farms, turkey slaughterers, and urban residents (Stobberingh et al., 1999). Fecal samples were taken from individual farmers, turkeys, slaughterhouse workers, and residents to be tested for VRE levels. Although one farmer and his flock shared an indistinguishable VRE strain, showing that animals and humans may carry the same clone, an additional finding of interest was that the strains were also resistant to quinupristin-dalfopristin. This antibiotic is a combination of streptogramins B and A and has been therapeutically used in human medicine in The Netherlands.

In 1999, owing to public and political pressure to decrease the amount of antibiotics used in food animals, the Danish poultry industry and swine industries voluntarily discontinued the use of antimicrobial growth promoters (AGPs). As a result of discontinuing AGPs, poultry producers have seen an increase in leg and skin problems (Casewell et al., 2003). The Danish pork industry has also seen negative impacts in mortality and weight gains. Although an overall reduction in antibiotic use has been observed, Danish veterinarians have resorted to using therapeutic antibiotics more regularly to combat increasing morbidity rates (Casewell et al., 2003).

Classes of Antibiotics

The antibiotics are grouped into classes based on chemistry, mode of action, and other properties. Penicillins are an example of a β-lactam antibiotic that works by inhibiting bacterial cell wall synthesis. This class is grouped into several subclassifications based on their ability to cross the cell wall and adhere to the penicillin binding proteins (PBPs) (Howland and Mycek, 2006). The subclassifications are as follows: β-lactamase-sensitive penicillins, β-lactamase-resistant penicillins, β-lactamase-sensitive penicillins with extended spectra, β-lactamase-protected penicillins, and carbapenems (Bush et al., 1995). Bacteria may become resistant to penicillins by altering PBPs, decreasing the cell wall permeability, or by producing β-lactamase enzymes.

Cephalosporins, also beta-lactam antibiotics, interfere with bacterial cell wall synthesis and are commonly used in human medicine (Howland and Mycek, 2006;

Tenover, 2006). One way to categorize cephalosporins is based on when they were discovered and their spectrum. There are four categories of cephalosporins: first-generation, second-generation, third-generation, and fourth-generation. The first-generation cephalosporins are very effective against Gram-positive bacteria, but less effective against Gram-negative bacteria (Williams et al., 2001). The second, third, and fourth generations of cephalosporins are effective against both Gram-positive and Gram-negative bacteria. Bacteria may become resistant to cephalosporins by altering the permeability barrier, changing the structure of the binding site, or by producing enzymes that destroy the drug (cephalosporinases or beta-lactamases).

Aminoglycosides inhibit protein synthesis in aerobic Gram-negative bacilli. Resistance to aminoglycosides may develop by altering the ribosomal binding site or through a lack of porin channels to transport the drug across the cell membrane. Two other antibiotic classes that inhibit protein synthesis by binding to the bacterial ribosome are the macrolides and tetracyclines (Tenover, 2006).

Antibiotics belonging to the tetracycline class may act on bacteria by inhibiting protein synthesis (Tenover, 2006). Since bacterial ribosomes are structurally different than those found in eukaryotic cells, the tetracyclines may selectively effect the bacterial growth in a host. The bacteria also may become resistant to tetracyclines by developing efflux pumps that eject the antibiotic before it reaches the target site.

Sulfonamides act as competitive inhibitors by binding to dihydropteroate synthetase and inhibiting folic acid synthesis (Howland and Mycek, 2006). In order to

overcome the competitive inhibitor, bacteria may overproduce para-aminobenzoic acid, or else alter the enzyme binding site. The Gram-negative bacteria, such as *E. coli*, may transfer resistance via plasmids; plasmid-mediated sulfonamide resistance is often linked to the use of ampicillin and tetracycline (Bean et al., 2005).

Quinolones affect the bacterial DNA replication process by targeting the DNA-gyrase enzyme and disrupting the DNA structure. Bacteria develop resistance by changing the enzyme target site (Poirel et al., 2005).

Future Prevention

Most research has indicated that the critical risk factor for resistance is antibiotic use. Currently, the FDA encourages food animal producers to create an antibiotic recording system. Although a mandatory reporting system is not in place, the U.S. National Pork Board has taken steps to educate pork producers on how to safely, effectively and efficiently use antibiotics (National Pork Board, 2007). One program is the Pork Quality Assurance Program (PQA) that was created in 1989 to improve management practices and increase awareness of food safety. The PQA program is a voluntary program, but most packers required that producers complete PQA level III certification as a pre-requirement for purchase of slaughter hogs. In June 2007, the PQA-Plus program was launched, which includes extensive animal welfare education for the producers and on-site assessment. On farms that have had producers complete the PQA Plus program, random audits (through the lottery system) will be conducted by a third party. PQA Plus recertification and on-farm assessments will be repeated at least

every 3 years. The PQA and PQA-Plus programs have introduced important principles and guidelines that in the future are expected to be observed by all U.S. pork producers.

Although public attention has been focused on antibiotic use in food animals, the human health care system must also accept responsibility. In the United States, patients expect and request antibiotics in cases where they may not be appropriate such as viral infections, both of which suggest a need for better patient education programs. The positive effect of patient education programs was realized in a 2001 pediatrics study. Parents of children under 4 years of age participated in community-wide educational programs focused on appropriate antibiotic use. The population that participated in the programs saw a decline in parents who expected but did not receive an antibiotic for their child and also a decrease in the percentage of parents who changed physicians as a result of not receiving an antibiotic (Trepka et al., 2001). Whether this decline in usage translates into a decline in levels of resistance was not, however, determined. Healthcare providers also need to curtail antibiotic use and only prescribe when needed, not simply to placate or pacify the patient. Unnecessary prescribing was seen in a study conducted in Kentucky, which found that 60% of patients seen for the common cold were prescribed an antibiotic (Mainous et al., 1996). Since the majority of antibacterial agents are prescribed in the community setting (an estimated 70%), it is extremely important that health care providers and patients responsibly prescribe and use these agents (Carrie and Zhanel, 1999).

The use of antibiotics in humans and animals and the effects on resistance levels is an international public health concern. The potential for transfer of antibiotic–resistant bacteria from food animals to humans is an issue that must be better studied in order to improve on-farm practices, decrease the level of resistance, and reduce risks to both animal and human health. This research project will address the association of antibiotic use with the prevalence of AR bacteria in a semi-closed and integrated population of humans and animals.

CHAPTER III

MATERIALS AND METHODS

Human Study Population

The aggregate cohorts of humans, housed in 19 geographically separate units, represented the study population for this project because the monthly antibiotic dispensing records were accessible and there was relatively limited human movement in and out of, as well as and across units. In each of the 19 units, multiple manhole samples from which to draw monthly wastewater (fecal matter) samples were strategically selected. These manholes were selected based on location, to represent (where appropriate) relationships to hospitals, kitchens, lavatories draining various housing dormitories, and agricultural worker housing facilities. Fecal samples arising from human wastewater collection are aggregated samples that cannot be traced to any individual.

Antibiotic Records

Typically, antibiotics were dispensed in the human population either by physicians at unit clinics or physicians at in-patient hospitals; the former either requiring patients to report directly to the clinic each day for dispensed medication or else permitting the patient to carry the antibiotic on their person. Each script was written, entered into a centralized computer system, filled by the central pharmacy, and then shipped to the unit within 24 hours (see Figure 1, Appendix A). The antibiotic class,

length of usage, and daily dose was logged into the mainframe computer. We requested the utilization records (aggregated across prescriptions, within drug code, by unit and housing cohort and with patient identifiers stripped from the records) for all medicines with any of the specified antibiotics as the active ingredient. These medicines were assigned identification numbers by the central pharmacy. Since there are numerous different medications, we requested a query of the mainframe computer for antibiotics known to have been used between January 2004 – January 2007. An example of such a search is as follows: erythromycin has the pharmacy identification code of 00064-3000. We requested dosage information, length of treatment, route of administration, major drug class, and finally, records concerning unused medications returned to the pharmacy by unit clinics/hospitals during the noted timeframe.

The formula for mean monthly dosages (MMDs) in the human population was as follows:

$$MMD_H = \frac{\text{total amount of antibiotic used (g)}}{[\text{total number of population at risk in a category (i.e., animal worker vs. non-animal worker) in the unit} \times \text{average body mass of a person at risk (kg)}] \times 1 \text{ month}}$$

The average body weight of a person at risk was taken from a 2004 study conducted by the U.S. Department of Health and Human Services. During the period of 1999-2002, the mean mass of men in the United States over the age of 20 was 86 kg (Ogden et al., 2004).

For analytic purposes, we grouped the antibiotics by class and used the aggregate data by month. As explanatory variables, these were treated first as a ratio relating the total mass (g) of an antibiotic utilized in a month at any given unit to the number of humans housed in the unit for that month.

A secondary coding of these aggregated antibiotic usage data related the mean monthly dosage (MMDs) per category of population at risk (agricultural worker or nonagricultural-worker), of an antibiotic class, relative to the overall human population in that category. The first ratio spread the effect of antibiotic selection pressure across the entire population, whereas the latter ratio spread the effect of antibiotic selection pressure across the category of population at risk that were prescribed the product. While both ratios are potentially correlated to some degree, it is possible that a relatively few individuals received a large proportion of the antibiotics, such that the selection pressure was not spread across the entire population's enteric flora.

Swine Study Population

There were 12 units that housed swine, with very limited movement of breeding swine into the system, and no pork products leaving the system except for waste byproducts after slaughter. A very small percentage of nursery/grower/finisher swine

were sold and exited the system due to poor growth performance. Monthly floor fecal samples were collected from the aggregate swine population at each unit (5 farrow-to-finish units and 7 grower-to-finish units). Antibiotics were distributed to the swine through the feed supply or directly administered via parenteral or oral routes (see Figure 2, Appendix A). We considered the feedgrade and injectable antibiotics separately for analysis. There was no formal antibiotic reporting system for actual use or administration in the food animals; therefore, we utilized the monthly listing of feedgrade antibiotics used (via formulations) and monthly antibiotic purchasing/dispensing records. The mean monthly dosages (MMDs) for swine were calculated as follows:

$$MMD_S = \frac{\text{total amount of antibiotic used (g)}}{\begin{array}{l}[\text{total number of pigs in a unit} \times \text{average body mass of a pig (kg)}] \\ \times \text{ 1 month}\end{array}}$$

We also considered the MMDs according to the swine categories: grower/finisher, nursery pigs, gestating and farrowing females and piglets[2], and boars; each with the average body mass adjusted accordingly. The average body mass for each

[2] The figure heading 'Farrowing pigs' and table heading 'Farrowing' includes gestating and farrowing females and piglets.

swine category was estimated by an experienced veterinarian from Texas A&M University College of Veterinary Medicine and Biosciences.

Sampling Method

The trained environmental compliance staff member for each designated unit directly supervised the monthly wastewater sample collection (February 2004 through January 2007 inclusive). Following collection, samples were stored in a refrigerator until the technician arrived for the scheduled pickup. Our contracted laboratory courier service transported samples to a central facility, stored them refrigerated at 4ºC, and then shipped each of the samples in a 100 ml sterile container to the USDA-ARS facility in College Station, TX for further processing.

A swine veterinarian from the College of Veterinary Medicine and Biomedical Sciences at Texas A&M University collected at least thirteen (range: 13-25) aggregated monthly swine fecal samples from each of the five farrow-to-finish units. In addition, at least four samples (range: 4-8) and two samples (range: 2-5) were collected each month from six, and one, grower-finisher units, respectively (7 units total). Each time the boar/gilt isolation site was occupied (about 3 times per year), individual (boar) and aggregate (gilt) sampling was performed. These samples were held in iced coolers and transported to USDA-ARS laboratories in College Station, TX for analysis.

Laboratory Analysis

Once the wastewater and fecal samples arrived at the laboratory they were logged and recorded by unit, manhole (for swine: pen number and production category), and date. Each original wastewater sample was thoroughly mixed and then divided into five 5 ml tubes as follows: three of the tubes received 4 ml of sample aliquot, in addition to being pre-filled with 1 ml of glycerol. The remaining 38 ml of sample were centrifuged for 20 minutes at 3200 rpm and the supernatant removed. The remainder was divided into two 5 ml vials. All five 5 ml tubes were frozen at -72°C. Each swine fecal sample was dispensed into five 5 ml tubes as follows: three of the tubes received 1 ml of glycerol and 4 ml of the fecal sample. The two remaining 5 ml tubes were filled with 5 ml of the swine fecal sample, and all five 5 ml tubes were frozen at -72°C.

Later, a single wastewater vial (with glycerol) was thawed in a 37°C water bath and subjected to enrichment and selective growth as follows. After thawing, 1 ml of sample was mixed with 9 ml of tryptic soy broth (TSB) and incubated at 37.5°C for 24 hours. Following incubation, 0.1 ml of the product was streaked onto CHROMagar-E.coli™ (DRG International, Mountainside, NJ) agar and Difco™ mEnterococcus agar (ME) (Becton Dickinson, Sparks, MD). The presumptive *E. coli* isolates were further incubated at 37.5°C for 24 hours, and the *Enterococci* were incubated at 45°C for 48 hours. Following incubation, a single *E. coli* colony (blue, smooth colony) was chosen to streak to a sheep-blood agar plate and was then incubated at 37.5°C for 24 hours. After incubating the *Enterococci for* 48 hours, a single colony (dark purple with a gold

sheen) was chosen to streak a blood agar plate and incubated at 37.5°C for 24 hours. The same two procedures for cultivating *E. coli* and *Enterococci* were followed for the swine fecal samples, except that the samples were not subjected to pre-enrichment with TSB.

Phenotypic Resistance Characterization

E. coli were tested for antibiotic susceptibility using microbroth dilution where minimum inhibitory concentrations (MIC) against 15 antibiotics (see Table 1, Appendix B) were determined. The *Enterococcus* spp. were also tested for antibiotic susceptibility using microbroth dilution where MICs against 17 antibiotics were determined (see Table 2, Appendix B). MIC values were interpreted according to prescribed Clinical Laboratory Standards Institute methods (formerly NCCLS) (National Committee for Clinical Laboratory Standards, 1999). Testing was completed on the Sensititre™ (Trek Diagnostics Inc., Cleveland, OH) 2003 NARMS test panel designed by the National Antimicrobial Resistance Monitoring System (NARMS), a jointly funded project of CDC, USDA and FDA.

Statistical Analysis

The antibiotic data were aggregated by month and descriptive statistics for each unit and host-species were calculated. In addition, appropriate ratios for antibiotics utilized in a month at any given unit and mean monthly dosages (MMDs) and dose categories were calculated for both host species. Histograms were constructed to illustrate the MMDs across all swine and human units, respectively. A few of the

antibiotics studied were used very sparingly in the study populations; these antibiotics were assessed further on a use versus nonuse basis. Those antibiotics were ceftiofur, tylosin, and tilmicosin in the swine population, and ciprofloxacin in the human population.

In order to assess the effect of MMDs on tetracycline resistance in the *E. coli* isolates in the swine study population, the MMDs were considered in three categories based on the histogram of distribution for the MMDs. The majority of the swine were not prescribed chlortetracycline; the isolates collected from these swine were considered in the zero-use category. The isolates collected from the swine prescribed ≤0.05 MMD were grouped together in the second category, and all isolates from swine prescribed >0.05 MMD were grouped in the third category.

When analyzing the tetracycline resistance in *E. coli* isolates for the human tetracycline class during the study period, the use was divided into 4 separate groups. The first group consisted of the isolates from the units prescribed MMDs ≤ 0.001 (g/population at risk kg), the second group were all isolates with MMDs > 0.001 and ≤ 0.002 (g/population at risk kg), the third group consisted of MMDs >0.002 and ≤ 0.004 (g/population at risk kg) and the fourth group were all isolates > 0.004 (g/population at risk kg).

Based on the histogram of the distribution of MMDs for the sulfonamide class in the human population, the MMDs were analyzed by 4 categories. The first category consisted of isolates from units with MMDs of 0 (g/population at risk kg), the second

category had MMDs >0 and ≤ 0.002 (g/population at risk kg), the third category had MMDs > 0.002 and ≤ 0.004 (g/population at risk kg), and the final category had MMDs > 0.004 (g/individual at risk kg).

The MMDs were grouped into 7 categories for the human penicillin class use during the period of January 2004 – January 2007. The categories are as follows: category 1 consists of isolates from units with MMDs of ≤ 0.001 (g/population at risk kg), category 2 with MMDS of > 0.001 and ≤ 0.002 (g/population at risk kg), category 3 with MMDs of > 0.002 and ≤ 0.003 (g/population at risk kg), category 4 with MMDs of > 0.003 and ≤ 0.004 (g/population at risk kg), category 5 with MMDs of > 0.004 and ≤ 0.005 (g/population at risk kg), category 6 with MMDs of > 0.005 and ≤ 0.007 (g/population at risk kg) and category 7 with MMDs of > 0.007 (g/population at risk kg).

A histogram illustrating the MMDs for erythromycin use from January 2004 – December 2004 was constructed. Based on the histogram, the MMDs for erythromycin in the human population were categorized into 3 categories: the first category consisted of isolates from units that prescribed MMDs 0 (g/population at risk kg), the second category of MMDs > 0 and ≤ 0.001 (g/population at risk kg), the third category of MMDs > 0.001 (g/population at risk kg).

The MMD categories for the penicillin class use during January 2004-December 2994 were obtained from a constructed histogram that illustrated the MMDs of penicillin class across all units. The three categories are as follows: category 1 consisted of isolates from units with MMDs of ≤ 0.002 (g/population at risk kg), category 2 of

MMDs of > 0.002 and ≤ 0.004 (g/population at risk kg), category 3 of MMDs of > 0.004 (g/population at risk kg).

The relationships between the prevalence of resistant bacteria (dependent variable) and host species, human occupational exposure to swine, unit location, sampling period (i.e. month), and the level of antibiotic use (independent variable) were assessed with generalized estimating equations (GEE) in a generalized linear model (GLM) model framework using a binomial distribution and a logit function in STATA® ver. 9.2 (StataCorp, College Station, TX). We built a model for each species resistance level based on antibiotic type and usage amount. When calculating the odds ratios for the swine population, the estimates were adjusted by swine production group, antibiotic MMD, and season. The estimates for the human population were adjusted by population at risk (i.e., non-worker or worker), MMD, and season. In addition, the seasonal variation among *E. coli* and enterococci isolates were evaluated in a GLM model in order to assess possible seasonality during the period of January 2004 – January 2007.

CHAPTER IV

RESULTS

E. coli *Isolates*

There were 5,929 (2,715 human and 3,214 swine) commensal *E. coli* isolated from the swine fecal and human wastewater samples collected from January 2004 – January 2007. During the period of 2004, the samples were analyzed monthly, but beginning in 2005 the samples were analyzed on a quarterly basis. In order to have a uniform sampling scheme, the 2004 samples were collapsed into quarterly groups according to season (see Table 3, Appendix B).

Tetracycline Resistance among Swine E. coli *Isolates*

Chlortetracycline (CTC) was the most commonly used antibiotic in the swine units from January 2004 – January 2007 (see Table 4, Appendix B). It was used in all of the swine production groups (boars, gestating and farrowing females and piglets, grower/finishers, and nursery) (see Figure 3, Appendix A). A histogram was constructed to illustrate the MMDs across all swine units during the study period (see Figure 4, Appendix A). By using the three categories of MMDs, a dose response relationship with MMDs of CTC for isolates from each unit/month was identified among the *E. coli* isolates for tetracycline resistance. The swine *E. coli* isolates were significantly ($P < 0.05$) more likely to exhibit resistance to tetracycline at the highest MMD category

(adjusted OR = 1.81) when compared to the isolates collected from swine groups with zero-use of CTC (see Table 5, Appendix B).

For this study, the chlortetracycline use and resistance level in each swine production group was also considered. The gestation and farrowing females and piglets were used as the reference group in the statistical analysis since they represented the smallest percentage of swine across the units being prescribed CTC (previously illustrated in Figure 3, Appendix A). The boars consisted of those in the farrow-finishing units (n=5 units) and purchased from outside sources (i.e., held at the quarantine unit). The grower/finisher (n=13) and nursery units (n=5; all at farrow-finishing units) were also considered in this study.

When considered by swine production group, the growers/finishers were prescribed the largest quantities of CTC during the period of January 2004 – January 2007. Although a smaller percentage of boars was prescribed CTC, *E. coli* isolated from the boars had statistically significant ($P < 0.05$) higher odds (adjusted OR = 1.51) of exhibiting tetracycline resistance than the reference swine category of gestating and farrowing females and piglets (see Table 6, Appendix B).

In order to assess possible temporal patterns concerning tetracycline resistance among *E. coli* isolates in the swine population, the isolates were grouped by season and year. No significant ($P > 0.05$) seasonality for the tetracycline resistance was observed during the study period (Figure 5, Appendix A).

Ceftiofur Resistance among Swine E. coli *Isolates*

Ceftiofur was used sparingly within the swine units and typically only in select pig production groups (see Figure 6, Appendix A). A histogram was constructed in order to evaluate the overall use of ceftiofur in the swine units from January 2004 – January 2007 (Figure 7, Appendix A). A very limited number of swine groups were prescribed ceftiofur; as a result, ceftiofur use in swine was considered in two categories: use versus nonuse (see Table 7, Appendix B).

A non-significant ($P = 0.282$) association between monthly ceftiofur use and resistance was found among the swine *E. coli* isolates. The *E. coli* from the swine that were housed in units during months that treatment with ceftiofur occurred had adjusted relative odds of 1.56 of exhibiting ceftiofur resistance (see Table 8, Appendix B).

When the *E. coli* ceftiofur resistance was considered by production groups, the *E. coli* from the nursery group had a significantly elevated ($P < 0.05$) adjusted odds ratio of 2.62 for exhibiting resistance (see Table 9, Appendix B). The gestating and farrowing females and piglets had the highest proportion of ceftiofur use in the system, and although the nursery pigs had zero ceftiofur use during the study period, the nursery units had a significantly increased relative odds of ceftiofur resistance. Ceftiofur was not used in the boars and a very low proportion of growers and finishers were prescribed ceftiofur.

The seasonal variation in ceftiofur resistance among *E. coli* isolates was evaluated in a (GLM) model in order to assess possible seasonality during the period of

January 2004 – January 2007. Although a higher proportion of ceftiofur resistant isolates qualitatively appeared in May 2004, no significant ($P > 0.05$) seasonal/cyclical trend was detected during the study period (see Figure 8, Appendix A).

Tetracycline Resistance among Human* E. coli *Isolates

The human therapeutic antibiotics considered in the tetracycline class were tetracycline and doxycycline. In order to evaluate the use of antibiotics belonging to the tetracycline class in the human study population, a histogram was created to illustrate the tetracycline class use over the entire study period (see Figure 9, Appendix A).

No significant ($P > 0.05$) association was identified for tetracycline MMD class and tetracycline resistance among *E. coli* in the overall human population (see Table 11, Appendix B). When the population was categorized into swine-worker versus non-worker groups, a nonsignificant ($P > 0.05$) difference in prevalence of tetracycline-resistant *E. coli* between the two groups was observed. From January 2004 – January 2007, the non-agricultural workers were prescribed a higher proportion of antibiotics in the tetracycline class than workers (see Figure 10, Appendix A); however, it was the *E. coli* isolated from agricultural workers' housing that exhibited a nonsignificant ($P > 0.05$) higher adjusted relative odds (OR = 1.19, P = 0.126) of tetracycline resistance when compared to the nonworkers (see Table 12, Appendix B).

No significant ($P > 0.05$) seasonal/cyclical trends were observed when the resistant *E. coli* isolates were analyzed on a quarterly basis in a (GLM) model (see Figure 11, Appendix A).

Sulfonamide Resistance among Human E. coli *Isolates*

Antibiotics belonging to the sulfonamide class were used only in the human study population (see Table 13, Appendix B). Based on the histogram of the distribution of MMDs for the sulfonamide class, the MMDs were analyzed by 4 categories (see Figure 12, Appendix A). For the overall human population, no relationship was found for sulfonamide class use and sulfisoxazole resistance (see Table 14, Appendix B).

The association of antibiotic use and resistance level was analyzed for the worker and non-worker groups (Figure 13, Appendix A). The non-workers had a significantly ($P < 0.05$) higher adjusted relative odds (OR = 1.67) of exhibiting sulfisoxazole resistance during the study period than the swine-workers (see Table15, Appendix B). Despite the qualitative observation (see Figure 14, Appendix A) that the proportion of sulfisoxazole-resistant isolates appear to be elevated during the month of November 2005, this increase did not represent a significant ($P < 0.05$) seasonal effect.

Ampicillin Resistance among Human E. coli *Isolates*

In order to evaluate the relationship between the use of antibiotics belonging to the penicillin class and ampicillin and amoxicillin/clavulanic acid resistance, a histogram was created to assess the MMDs over the study period (see Figure 15, Appendix A).

The majority of the isolates from the overall human study population were susceptible to ampicillin (see Table 16, Appendix B). No ($P > 0.05$) relationship was found for penicillin class use and ampicillin resistance in the overall human population.

The nonagricultural workers had a higher percentage of patients in the population at risk who were prescribed an antibiotic belonging to the penicillin class than the worker category (see Figure 16, Appendix A). When the relationship between the population at risk and ampicillin resistance was analyzed by a GEE equation, the nonworkers had a nonsignificantly ($P > 0.05$) adjusted higher odds (OR = 1.23) of harboring ampicillin resistant *E. coli* than the workers (see Table 18, Appendix B).

No significant seasonal/cyclical trend was observed for ampicillin resistant isolates during the study period (see Figure 17, Appendix A).

Amoxicillin/Clavulanic Acid Resistance among Human* E. coli *Isolates

The majority of the human *E. coli* isolates collected from January 2004-January 2007 were susceptible to amoxicillin/clavulanic acid (see Table 19, Appendix B). Based on the histogram of MMD for penicillin class use across all units, the MMDs were considered in 7 categories when analyzing the relationship of amoxicillin/clavulanic acid resistance and penicillin class antibiotic prescribing practices.

There was no relationship observed for penicillin class use and amoxicillin/clavulanic acid resistance during the study period (see Table 20, Appendix B). As previously discussed, a higher proportion of the population at risk in the nonworker category were prescribed antibiotics belonging to the penicillin class than the population at risk in the worker category. The *E. coli* from the nonworkers had a nonsignificantly ($P > 0.05$) higher adjusted odds (OR = 1.16) of exhibiting resistance to amoxicillin/clavulanic acid than the workers (see Table 21, Appendix B).

No significant ($P > 0.05$) seasonal/cyclical trend was observed for amoxicillin/clavulanic acid resistance during the study period (see Figure 18, Appendix A).

Ciprofloxacin Resistance among Human E. coli *Isolates*

Ciprofloxacin was the only antibiotic in the flouroquinolone class that was used during the study period in the human population. The MMD categories were created based on the observation from the histogram of MMD across all units from January 2004-January 2007 (see Figure 19, Appendix A). The MMDs for ciprofloxacin use were considered on a use versus nonuse basis for analysis in a GEE model.

A very small proportion of the human *E. coli* isolates were resistant to ciprofloxacin (see Table 22, Appendix B). A nonsignificant ($P > 0.05$) relationship was observed for ciprofloxacin use and resistance during the study period. Although it was not statistically significant ($P < 0.05$), the *E. coli* from the population at risk in the units that prescribed ciprofloxacin had an adjusted higher odds (OR = 1.8, $P = 0.317$) of exhibiting resistance than the *E. coli* from the population at risk in units that did not prescribe ciprofloxacin (see Table 23, Appendix B).

A higher proportion of nonworker cohorts were prescribed ciprofloxacin than agricultural worker cohorts in the human study population (see Figure 20, Appendix A). A nonsignificant ($P > 0.05$) relationship with ciprofloxacin use within the unit and *E. coli* from the population at risk category was observed. *E. coli* from the population at risk in the worker category had a nonsignificant ($P > 0.05$) adjusted higher OR of 1.21

for ciprofloxacin resistance when compared to the nonworker category (see Table 24, Appendix B).

No significant ($P > 0.05$) season/cyclical trend was observed for ciprofloxacin resistance in the human *E. coli* isolates (Figure 21, Appendix A).

Enterococcus Spp.

There were 669 (323 human, 346 swine) commensal enterococci isolated from the swine fecal and human wastewater samples collected from January 2004 – December 2004. The samples were collected and analyzed by month.

Tylosin Resistance among Swine Enterococci *Isolates*

The majority of the enterococci isolates exhibited tylosin resistance (see Table 25, Appendix B). A histogram of the tylosin MMDs was constructed in order to evaluate the range of values across the swine units (see Figure 22, Appendix A). Tylosin use within the units was considered as use versus nonuse. A significant ($P < 0.05$) relationship between tylosin use in the units and tylosin resistance was found. Enterococci from the units that prescribed enhanced feed containing tylosin had a statistically significant ($P < 0.05$) higher odds (OR = 3.54) of exhibiting tylosin resistance than the swine units with no tylosin use (see Table 26, Appendix B).

Although the grower and finisher pigs were prescribed the largest amount of tylosin enhanced feed, the enterococci from the gestating and farrowing females and piglets had a significantly ($P < 0.05$) higher adjusted odds of tylosin resistance than the grower and finisher pigs (see Figure 23, Appendix A). The enterococci from the boar

and nursery units had a nonsignificantly ($P > 0.05$) higher adjusted odds (OR = 2.1, $P = 0.164$ and OR = 1.6, $P = 0.299$) of exhibiting resistance than the farrowing units (see Table 27, Appendix B).

No significant ($P > 0.05$) seasonal/cyclical trend was observed for tylosin resistance in the swine units during the study period (see Figure 24, Appendix A).

Erythromycin Resistance among Swine* Enterococci *Isolates

A very small number of the swine isolates were sampled from pigs prescribed tilmicosin enhanced feed (see Table 28, Appendix B). The MMDs for tilmicosin in swine were categorized as use versus nonuse based on the evaluation of the histogram which illustrated the MMDs of tilmicosin across all swine units (see Figure 25, Appendix A). No relationship between tilmicosin use and erythromycin resistance was found in the isolates from the overall swine population.

The nursery units were the only units that treated a few select pigs with tilmicosin (see Figure 26, Appendix A), but when the swine production categories were analyzed, the enterococci from the boar units had a statistically significant ($P < 0.05$) higher adjusted odds (OR = 3.5, $p = 0.016$) of erythromycin resistance than the farrowing units (see Table 29, Appendix B). The enterococci from the nursery had a slightly higher adjusted odds (OR = 1.91, $p = 0.147$) but it was not statistically significant ($P > 0.05$).

When the resistant isolates were analyzed for seasonal/cyclical trend in the (GLM) model, no significant ($P > 0.05$) seasonal/cyclical trend was observed for erythromycin resistance during the study period (see Figure 27, Appendix A).

Erythromycin Resistance among Human* Enterococci *Isolates

The majority of the human *Enterococcus* spp. isolates were susceptible to erythromycin (see Table 30, Appendix B). In order to evaluate the relationship between erythromycin use and resistance, a histogram was constructed to show the MMDs of erythromycin across the human units (see Figure 28, Appendix A).

No relationship between erythromycin use and resistance in the total human population was found (see Table 31, Appendix B). A higher proportion of nonagricultural workers were prescribed erythromycin than the agricultural workers (see Figure 29, Appendix A). The enterococci from the non-workers had a nonstatistically significant ($P > 0.05$) higher adjusted odds (OR = 3.79, $p = 0.056$) of harboring erythromycin resistant *E. coli* than workers (see Table 32, Appendix B).

No significant ($P > 0.05$) seasonal trend was observed for erythromycin resistance in the enterococci isolates from January 2004 – December 2004 (see Figure 30, Appendix A).

Penicillin Resistance among Human* Enterococci *Isolates

The majority of the enterococci isolates were obtained from units where antibiotics belonging to the penicillin class were prescribed, but only a small proportion of those isolates were resistant to penicillin (see Table 33, Appendix B). The MMD

categories were obtained from a constructed histogram that illustrated the MMDs of penicillin class across all units (see Figure 31, Appendix A).

There was no relationship between penicillin resistance and use of antibiotics belonging to the penicillin class (see Table 34, Appendix B). A higher proportion of nonworkers were prescribed antibiotics belonging to the penicillin class than the swine workers (see Figure 32, Appendix A). The enterococci from the nonworkers had a nonsignificant ($P > 0.05$) higher adjusted odds (OR = 1.32, $p = 0.531$) of exhibiting penicillin resistance than workers (see Table 35, Appendix B).

There was no significant ($P > 0.05$) seasonal trend observed for penicillin resistance during the study period (see Figure 33, Appendix A).

CHAPTER V

DISCUSSION

In the present study *E. coli* and *Enterococcus* spp. were selected as surveillance organisms. These commensal bacteria are generally considered to be good indicators due to their ability to serve as a reservoir for resistance genes and to transfer resistance to pathogenic bacteria (Witte, 1998; Winokur et al., 2001; Hayes et al., 2004; Scott et al., 2005). Also, *E. coli* and *Enterococcus* spp. are present in human wastewater and swine fecal samples in high concentrations and may be easily isolated (Huycke et al., 1998; Manero et al., 2006).

Swine Resistance

When the *E. coli* swine data were analyzed, relationships between antibiotic use and resistance within swine categories were observed. The relationship between tetracycline resistance and MMD illustrated a dose-response association, with adjusted odds ratios (OR) of 1.20 and 1.81 ($P < 0.05$) for second and third categories of MMDs for chlortetracycline (CTC) use in medicated feed. Odds ratios were adjusted for confounding effects of swine production group and season. The variability in tetracycline resistance was somewhat explained by the variable levels of CTC use in feed, though resistance did not disappear in the absence of treatment. This finding is consistent with other studies which found high levels of tetracycline resistant *E. coli* isolated from swine (Teshager et al, 2000; Schroeder et al, 2002b; Dewulf et al, 2007;

Stine et al, 2007). Stine et al. (2007) studied the distribution of tetracycline resistance among bacteria isolated from a swine facility with a history of continual use of tetracycline-enhanced feed. Of the *E. coli* isolates tested, 77% were resistant to tetracycline. The present study demonstrated a baseline level of resistance among untreated animals at 81.7%. The researchers in the Stine et al. (2007) study also found a significant difference in tetracycline resistant isolates present between isolates from feed hogs, houses, or lagoons and water or soil. This indicates the increased chance of having resistant bacteria from exposure to swine and housing facilities and the need to obtain samples in order to assess this risk as is done in the present study.

When broken down into groups, the boars had the highest relative odds of harboring tetracycline–resistant *E. coli*. Although the grower and finisher units had the highest levels of chlortetracycline use, the boars had the highest odds of exhibiting resistant bacteria. This finding is not in agreement with studies which found a higher level of resistance in younger pigs (Langlois et al., 1988; Matthew et al., 1999). Langlois et al. (1988) sampled, over a 20 month period, fecal matter from antibiotic-free pigs located in farrowing houses, finishing units, or on pasture. A higher proportion of tetracycline-resistant isolates were obtained from pigs 6 months of age or less. However, in this present study, the oldest pigs in the system had the highest level of tetracycline resistance. This may partially be due to purchasing boars from outside sources where the use of antibiotics is more prevalent. By grouping the purchased boars with those housed in the system for longer periods of time, we had no antibiotic

treatment history for the purchased boars. This may explain the higher level of resistance found in the boar population. The boars are also in the system (up to 5 years old) longer than the pigs; typically the pigs are in the system (from birth to finish) an average of 6 months before going to slaughter. Over this time, the boars have bred hundreds of sows and have been exposed to resistant bacteria in the manure and other body fluids whereas the finisher pigs have been exposed to at most 300 contemporary pigs during their lifetime.

Ceftiofur was rarely used in the swine units; it was only used in units containing gestating and farrowing females and piglets. A non-significant relationship between ceftiofur resistance and prescribing (OR = 1.56, $P > 0.05$) was found. Ceftiofur was used to treat conditions in lactating sows that result in milk reduction and therefore affected growth performance in the piglets. This antibiotic was also used to treat unweaned piglets for diarrheal diseases caused by *E. coli*. The gestating and farrowing females and piglets had the highest proportion of ceftiofur use in the system, but the *E. coli* from the nursery units had a higher OR of 2.61 ($p < 0.05$) for exhibiting ceftiofur resistance than the farrowing units. The higher odds in nursery pigs may result from piglets that were directly exposed to ceftiofur via injection or indirectly through treatment of their sow before moving into the nursery units. In the swine *E. coli*, the baseline level of resistance to ceftiofur was 2.24%. This finding is in agreement with another study that was conducted on pigs from Spanish slaughterhouses (Teshager et al., 2000). The fecal samples were collected from swine directly after slaughter and tested

for antibiotic susceptibility. A low level of resistance to cephalothin was found (a separate antibiotic belonging to the cephalosporin class was used in the swine population). The researchers only surveyed the resistance levels in a selected pig population without knowledge of corresponding antibiotic use. This present study evaluated the antibiotic resistance in each swine category and also considered the amount of antibiotics used in each production category.

Ceftiofur has been authorized for use in food animals for a relatively short period of time (first authorized in cattle in 1988), but plasmid-mediated resistance transfer between bacterial species has been reported (Winokur et al. 2001; Philippon et al., 2002; Batchelor et al., 2005). Winokur et al. (2001) identified isolates of *E. coli* from bovine, swine, and humans that have the same CMY-2 B-lactamase. Identical CMY-2 plasmids were also observed in *E. coli* and *Salmonella* isolates. These findings suggest a transfer of resistance between bacterial species. For this present study, only cephalosporin resistance was considered for the swine population; however, since the antibiotic utilization was calculated for the swine this important risk factor could be evaluated. Although ceftiofur resistance has developed in the food animal population, in this present study the baseline level of resistance is still very low.

When the enterococci isolates were analyzed, a relationship between tylosin use and odds of tylosin resistance was observed. The relationship between tylosin resistance and use in medicated feed was illustrated with an adjusted odds ratio (OR) of 3.54 ($P < 0.05$). This finding is in agreement with a study conducted in swine in the European

Union from 1998 to 2000 which found an association between tylosin use as a growth promoter and prevalence of resistance (Bywater et al., 2005). After the first year of sampling, tylosin use as a growth promoter was discontinued. As a result, differences in the prevalence of resistant strains were observed between year 1 and year 2 of the study. Bywater et al. (2005) only studied the prevalence of tylosin resistance across the two year period without assessing the dosage of tylosin given to the swine before the discontinuation of tylosin. The boars and nursery units had higher odds of exhibiting resistance than the gestating and farrowing females and piglets, but the odds were not statistically significant. Although the grower and finisher pig units consumed the most tylosin medicated feed from January 2004-January 2007, those units showed statistically significant ($P < 0.05$) lower odds of having isolates with tylosin resistance than the farrowing units. The lower odds in the isolates from grower and finisher pigs may be explained by the short period of time the grower and finisher pigs were treated before slaughter and, as a result, the prevalence of resistant bacteria may be lower in the fecal matter.

No relationship was found for use of tilmicosin medicated feed and erythromycin resistance during the period of January 2004 – December 2004. When considered by swine production group, the isolates from boars had a statistically higher OR of 3.5 ($P < 0.05$) of erythromycin resistance than the pigs in the farrowing buildings, but the nursery units were the only units given the medicated feed. The isolates from nursery pigs only had slightly higher odds of erythromycin resistance. The higher level of erythromycin

resistance in isolates from boars may be explained by the length of time spent in the system along with the possibility of previous antibiotic use from outside sources before purchase into the system.

No seasonal trend was found for swine tetracycline, ceftiofur, tylosin, or erythromycin resistance. This finding is not in agreement with the theory proposed by Moro et al. (2000) that changes in temperature affect the level of resistant isolates in swine at production facilities (Moro et al., 1998; Moro et al, 2000). The researchers observed higher levels of resistant bacteria immediately following exposure to heat stress with a progressive drop in resistance after the exposure. According to their theory, a higher proportion of resistant isolates should have been observed during the summer months (May and August), but no significant increase of the proportion of resistant isolates was found in this present study.

Human Resistance

No relationship was found for tetracycline class use and tetracycline resistance in the overall human population, but when the population was categorized into worker/nonworker a modest nonsignificant ($P > 0.05$) difference between the two groups was observed. Although, in this thesis, the proportion of nonworkers who were prescribed antibiotics belonging to the tetracycline class was much higher than the proportion of workers, the workers had a nonsignificantly higher OR of 1.19 ($P > 0.05$) of tetracycline resistance than the nonworkers. This finding does not agree with a previous study that found a higher prevalence of tetracycline resistant bacteria in the

commensal flora of pig farmers (Aubry-Damon et al., 2004). Aubry-Damon et al. (2004) selected 113 pig farmers and 113 nonfarmers with no antibiotic use within the past month. Throat, nasal, and fecal swabs were obtained from the study participants only once during the study. Unlike this present study, Aubry-Damon et al. relied on retrospective estimates of antibiotic use and one-time samples. In this thesis, one possible explanation for the higher odds of resistance is that workers are in contact with more antibiotic resistant bacteria from the pigs. Workers may come into contact with swine feces while directly handling the pigs or cleaning the stalls. Also, the workers have contact direct contact with the antibiotics themselves during feed distribution to the swine. During the feed distribution, the workers may breathe dust that is laden with antibiotic residue and thus may be given a small daily dose of chlortetracycline.

The sulfonamides are commonly used to treat urinary tract infections caused by *E. coli.* When the sulfonamide class effect on sulfisoxazole resistance was considered by worker/nonworker categories, the isolates from nonworkers had statistically significant ($P < 0.05$) higher odds of sulfixazole resistance than the workers. This may be attributed to the higher percentage of nonworkers prescribed sulfonamides than workers. The population at risk in the worker category may have fewer chronic illnesses, also known as the 'healthy-worker' effect. The workers were also responsible for a much lower proportion of sulfonamide use than the nonworkers.

In the human study population no statistically significant ($P > 0.05$) relationship between penicillin class MMDs and ampicillin or amoxicillin/clavulanic acid resistance

was observed. The nonworkers were prescribed a higher percentage of those antibiotics belonging to the penicillin class than the workers. Not surprisingly, a higher odds of ampicillin and amoxicillin/clavulanic acid resistance were observed in the isolates from the nonworker category. A case-control study conducted by Hillier et al. (2007) in South Wales found a significant association of amoxicillin use and ampicillin resistance in patients with urinary tract infection. Hillier et al. (2007) sampled patients from local general practices and surveyed the patients on previous antibiotic use within the previous year. The population at risk with ampicillin-resistant *E. coli* were significantly more likely to have been prescribed ampicillin within the past year than the controls. The study considered the risk of developing resistance in response to antibiotic use on the individual level, whereas in this present study the risk was assessed at the aggregate level; that is, the denominator included all housed individuals, whether prescribed antibiotics or not. That said, our interest is at the ecological level.

Ciprofloxacin belongs to the fluoroquinolone class; flouroquinolones are commonly used to treat urinary tract infections caused by *E. coli*. *E. coli* may become resistant to flouroquinolones by mutating the target site and thus disabling the antibiotic's ability to attach to the bacteria, or by efflux pumps which eject the antibiotic out of the cell (McDonald et al., 2001). In this present study, no significant ($P > 0.05$) association between treatment with ciprofloxacin and resistance was observed and there was also not a statistically significant difference between worker and nonworker odds of ciprofloxacin resistance observed during the study period. The findings of this present

study are not in agreement with a study conducted in Barcelona, Spain (Garau et al., 1999). Garau et al. (1999), found recent exposure to ciprofloxacin to be significantly associated with ciprofloxacin resistance in the human study population. Although in this present study the majority of the nonworker population monthly cohorts were prescribed ciprofloxacin during the study period, no significant ($P > 0.05$) difference between the two groups was observed.

Traditionally, serious enterococcal infections have been treated with a combination of penicillins and aminoglycosides, but the emergence of resistance has led physicians to look for other treatments (Hodges et al., 1992). One mechanism that enterococci employ for resistance to penicillins is the overproduction of low-affinity penicillin-binding proteins (Murray, 1998). When the enterococci human isolates were analyzed no relationship between erythromycin and penicillin use and resistance was found for the period of January 2004-December 2004. The isolates from nonworkers had statistically significant ($P < 0.05$) higher adjusted odds of 3.58 of erythromycin resistance than the isolates from nonworkers which may be attributed to a higher percentage of use in nonworkers.

To the author's knowledge this is the first study to have looked at resistance in relation to antibiotic use within an integrated agri-food system. In the past, it has been difficult to establish a link between antibiotic consumption and the prevalence of resistance due to lack of antibiotic utilization records for human and swine populations in the study and also unlimited migration of study participants and lack of useful housing

cohort information. By conducting this study in a relatively closed population with known antibiotic consumption and some with direct exposure to swine treated with antimicrobials, we have been able to address several questions concerning the relationship between use and resistance. Also by considering the resistance and antibiotic use over a 3 year period at the same location we have provided insight into a possible seasonal/cyclical trend in antibiotic resistance levels. The study populations were unique due to limited movement out of the system and across units. Also the integration of the human and swine populations provided valuable insight into the possible links between occupational exposure and resistance. While an increased odds of tetracycline resistance was found in agricultural workers, a causal association cannot be reached at this time. Further study needs to be conducted to assess the resistance at intermediate breakpoints instead of only considering resistance versus susceptible outcomes.

Study Limitations

One of the limitations to the swine portion of the study was the antibiotic utilization records. Although monthly feed recipes were obtained from the feed mills in the system, the amount of antibiotic ingested by each pig was only an estimate. Each unit recorded the feed and amount that was received from the feed mill but no record of the amount of feed that was dispensed was kept. The production group prescribed an antibiotic and the duration of the treatment were estimated by experienced veterinarians from Texas A&M College of Veterinary Medicine and Biosciences. Therefore, it was

assumed that all feed was properly dispensed to the swine in each unit. Also, each pig was not individually weighed in order to calculate the MMDs. The average weight of a pig in each separate category was estimated by an experienced swine veterinarian from Texas A&M College of Veterinary Medicine and Biosciences. For the injectable antibiotics, only the records of the vials sent to the units were available for this study. Therefore, we assumed that all of the vials were used within the month they were sent to each unit.

For the human portion of the study, the swine workers and nonworkers were analyzed separately. The records of the population at risk categories only provided the worker/nonworker status. The various jobs for the workers were not provided, therefore all of the workers were considered as having contact with the swine if they were located in units with swine facilities. Another limitation to this study was the antibiotic utilization records. The records only recorded the amount dispensed from the central pharmacy and for the purposes of this study we assumed each individual took the entire prescription.

For the purposes of this thesis, only the outcome of resistant bacteria versus susceptible bacteria was considered. The breakpoints used when evaluating the phenotypic characterization for *E. coli* and enterococci were obtained from the Clinical Laboratory Standards Institute. Although this method provided insight into the prevalence of resistance, this may provide a limitation to this study by not considering the actual minimal inhibitory concentration values.

CHAPTER VI

CONCLUSIONS

Summary and Conclusions

Our study showed that risk of tetracycline, ceftiofur, and tylosin resistance in bacteria from swine is dependent on CTC, ceftiofur, and tylosin use, respectively. The swine production cycle effect on the risk of tetracycline, ceftiofur, tylosin, and erythromycin resistance is also important. The study indicated that risk of tetracycline resistance in human bacterial isolates may be associated with exposure to swine. Also, the temporal/seasonal effects on the risks of resistance for the antimicrobials studied in this project were not important.

This study provides a better understanding of the relationships between antibiotic prescribing practices at the ecologic level and the relative risks of transmission of resistant bacteria within host species in a vertically integrated agri-food system.

Recommendations for Future Studies

Resistant organisms are becoming increasingly common , and these resistant bacteria are responsible for increased morbidity and mortality as a result of treatment failures. Not only are resistant bacteria a problem in healthcare facilities, but also in the community. Cases with resistant bacteria are reported from sports teams, child daycares, and military personnel. A financial strain has been placed on the healthcare system with the added stress of infection containment in health facilities, costly use of antibiotics,

and development of new antibiotics for future use. There is an urgent need to better understand the transmission dynamics of resistant bacteria between food animals and humans. In order to achieve that goal, future studies should further assess antimicrobial usage in human and swine populations by obtaining drug utilization records instead of relying on rough estimates of usage. Also, records of the movement of humans across the categories of worker/nonworkers should be kept in order to track the exposure time to the swine. Finally, a study considering intermediate resistant breakpoints or actual minimal inhibitory concentrations of antibiotics would provide valuable insight for determining prevalence of resistance at intermediate levels.

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APPENDIX A

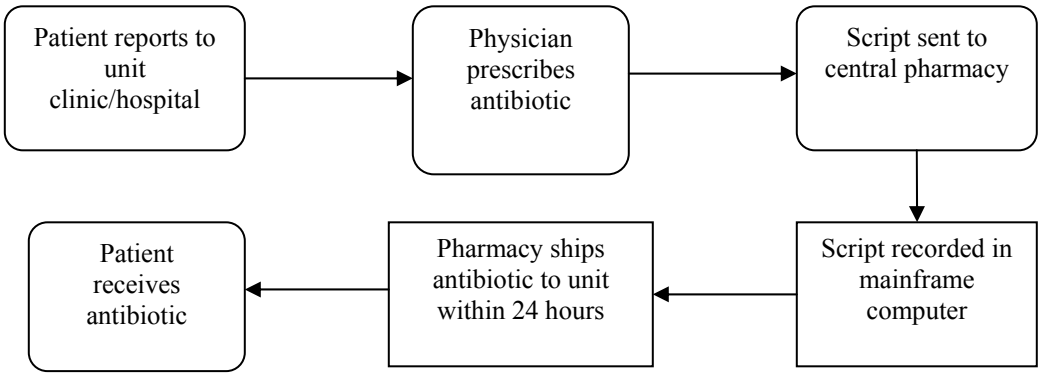

Figure 1. Antibiotic dispensing method within the human study population.

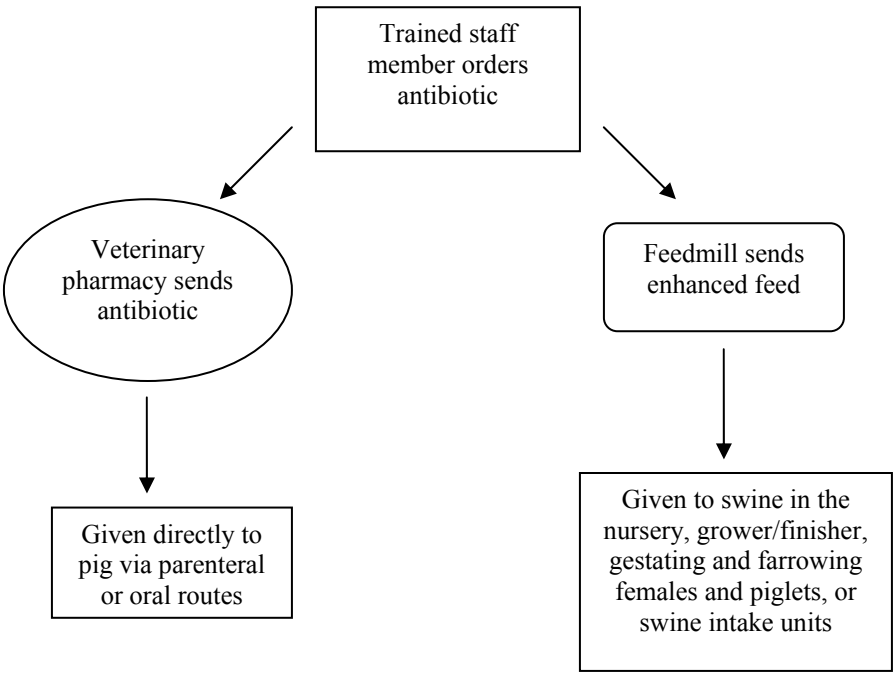

Figure 2. Antibiotic dispensing method within the swine study population.

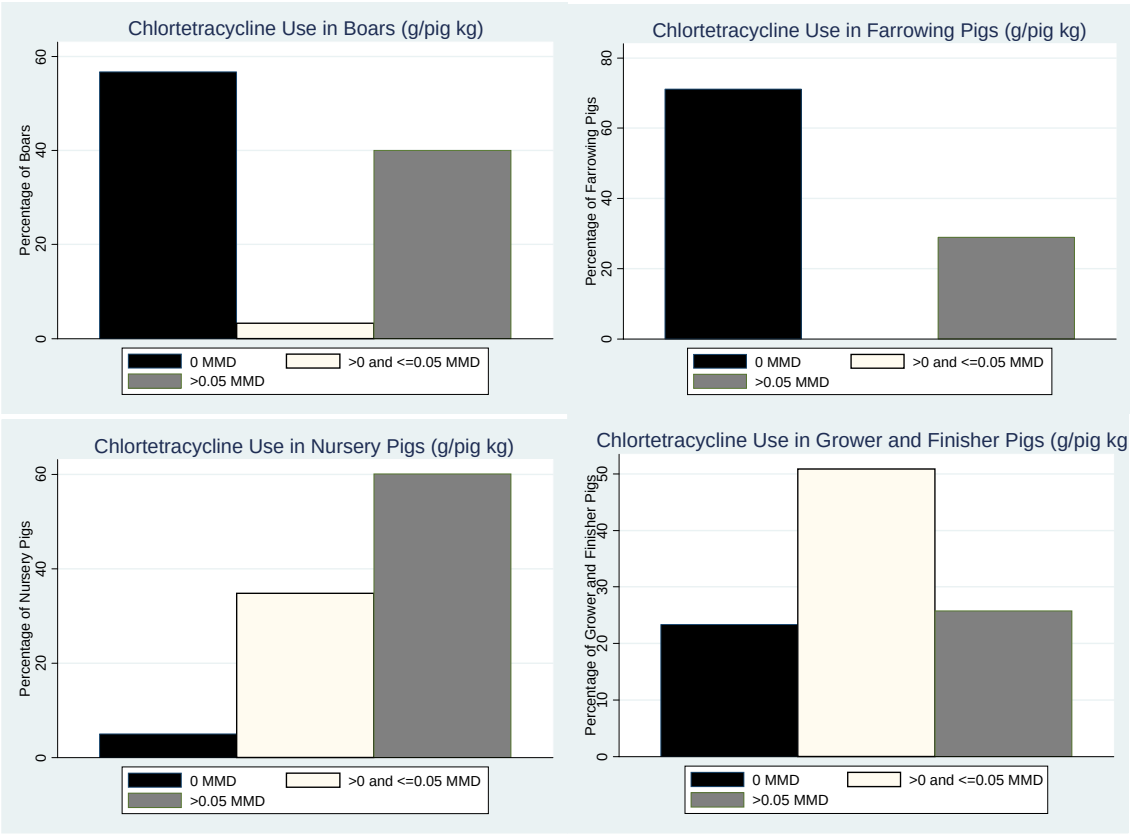

Figure 3. Chlortetracycline mean monthly dosages distributed among the swine production categories.

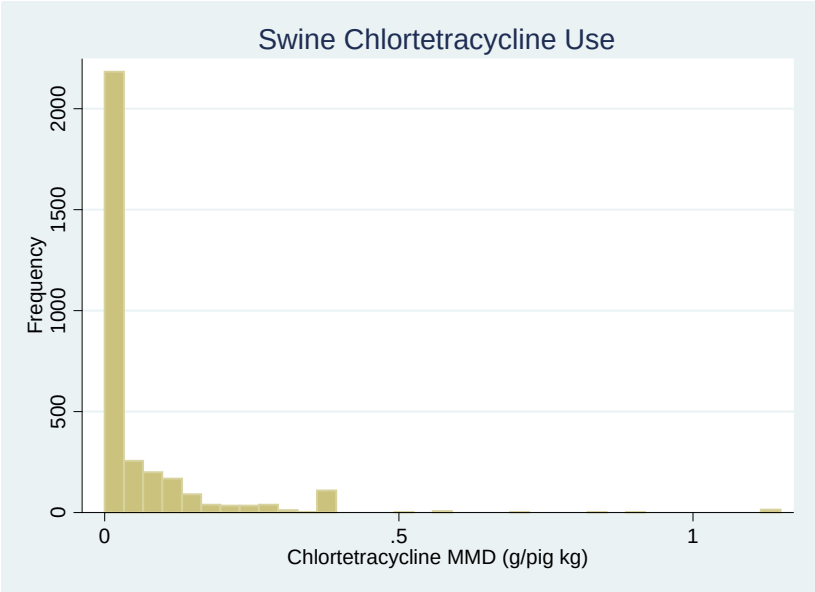

Figure 4. Chlortetracycline use across all swine units from January 2004-January 2007.

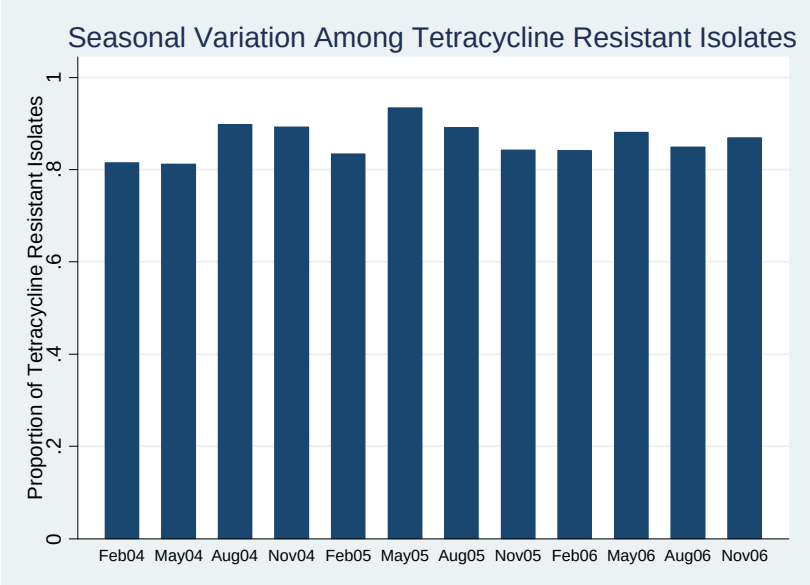

Figure 5. The proportion of swine tetracycline resistant *Escherichia coli* isolates collected from February 2004 – November 2006.

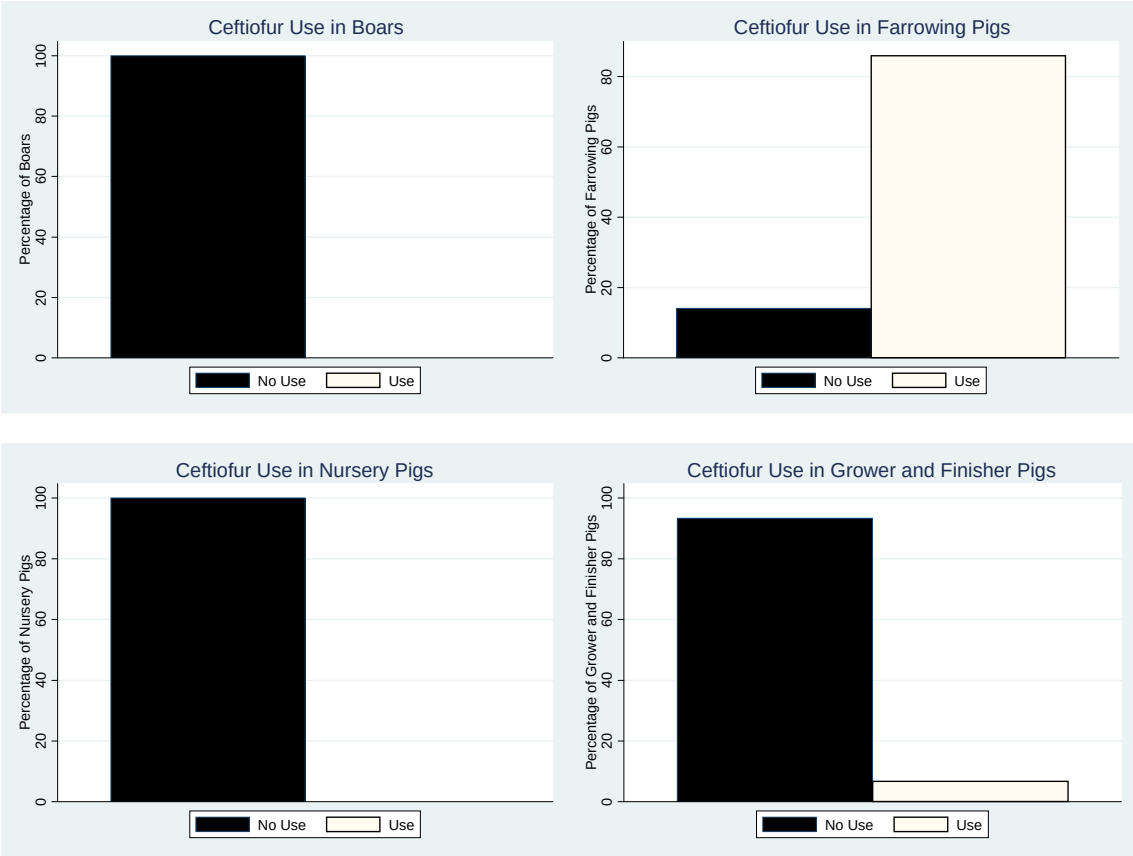

Figure 6. Ceftiofur use distributed among the swine categories from January 2004- January 2007.

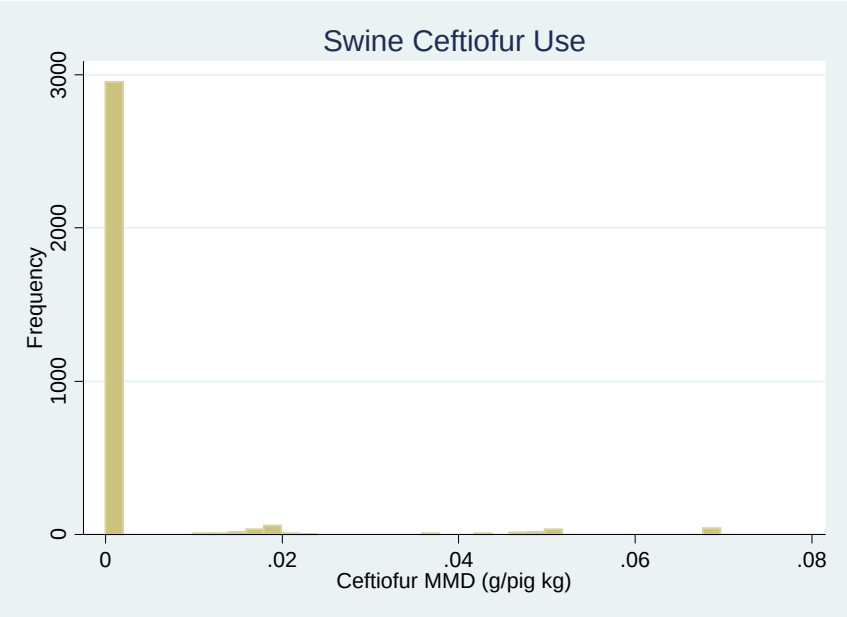

Figure 7. Ceftiofur use across all swine units from January 2004-January 2007.

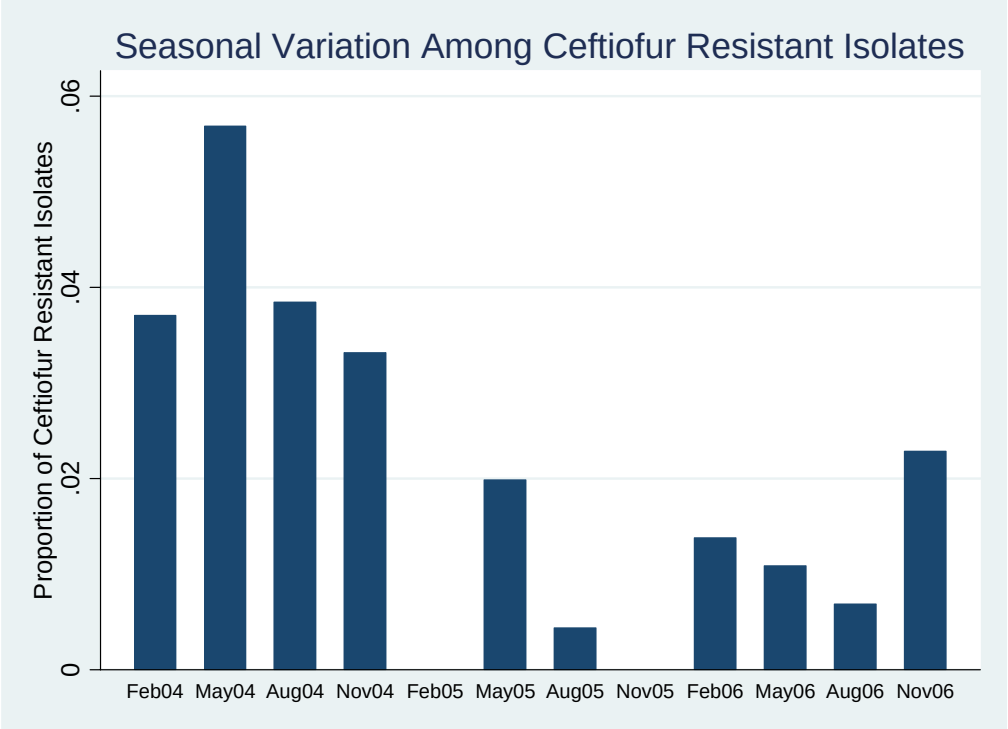

Figure 8. The proportion of ceftiofur resistant swine *Escherichia coli* isolates sampled from February 2004 – November 2006.

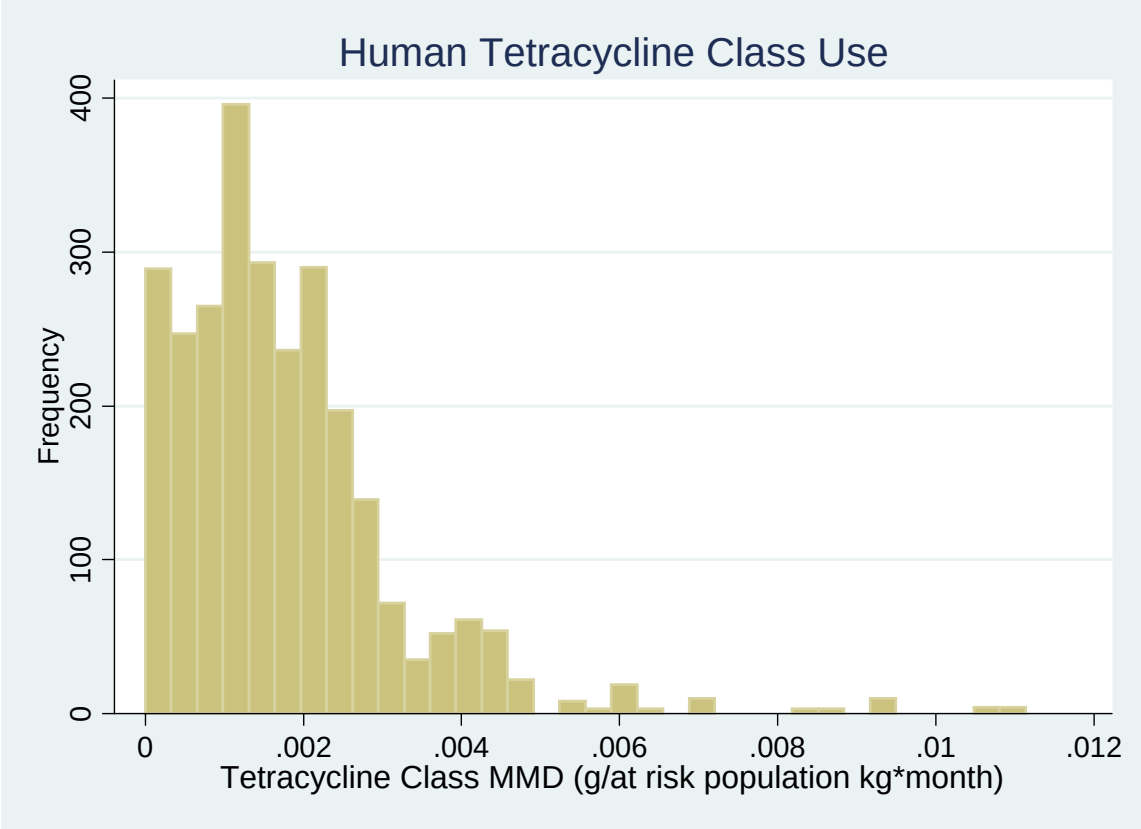

Figure 9. The human tetracycline class use across all units from January 2004-January 2007.

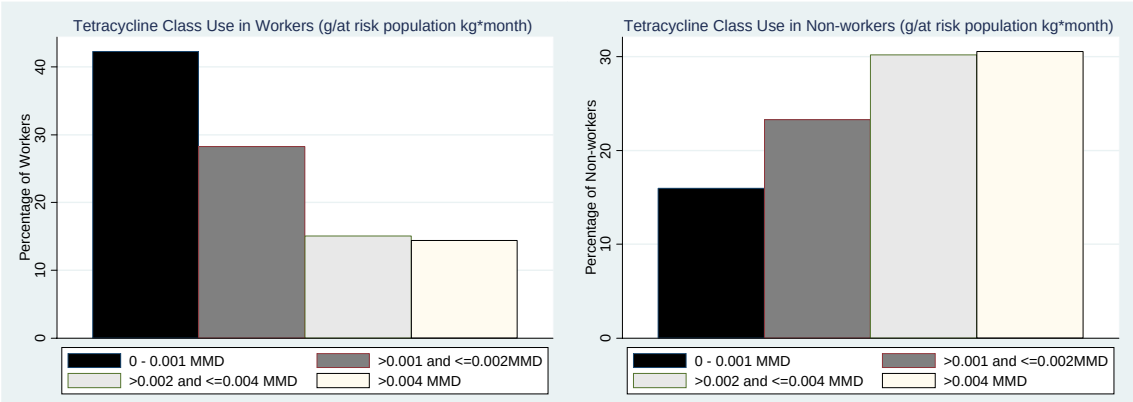

Figure 10. Tetracycline class use distributed among the human categories.

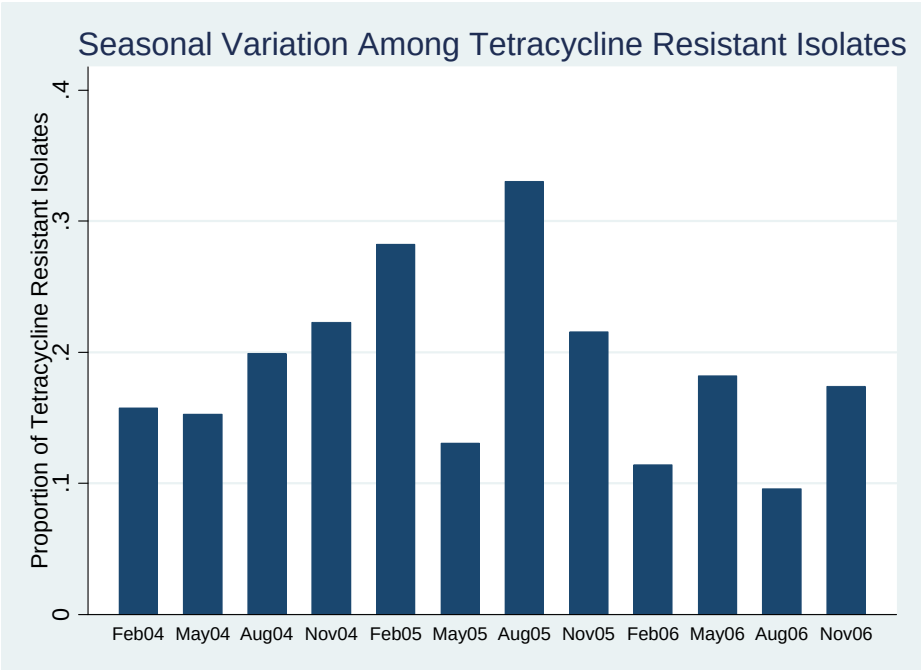

Figure 11. The proportion of tetracycline resistant human *Escherichia coli* isolates sampled from February 2004 – November 2006.

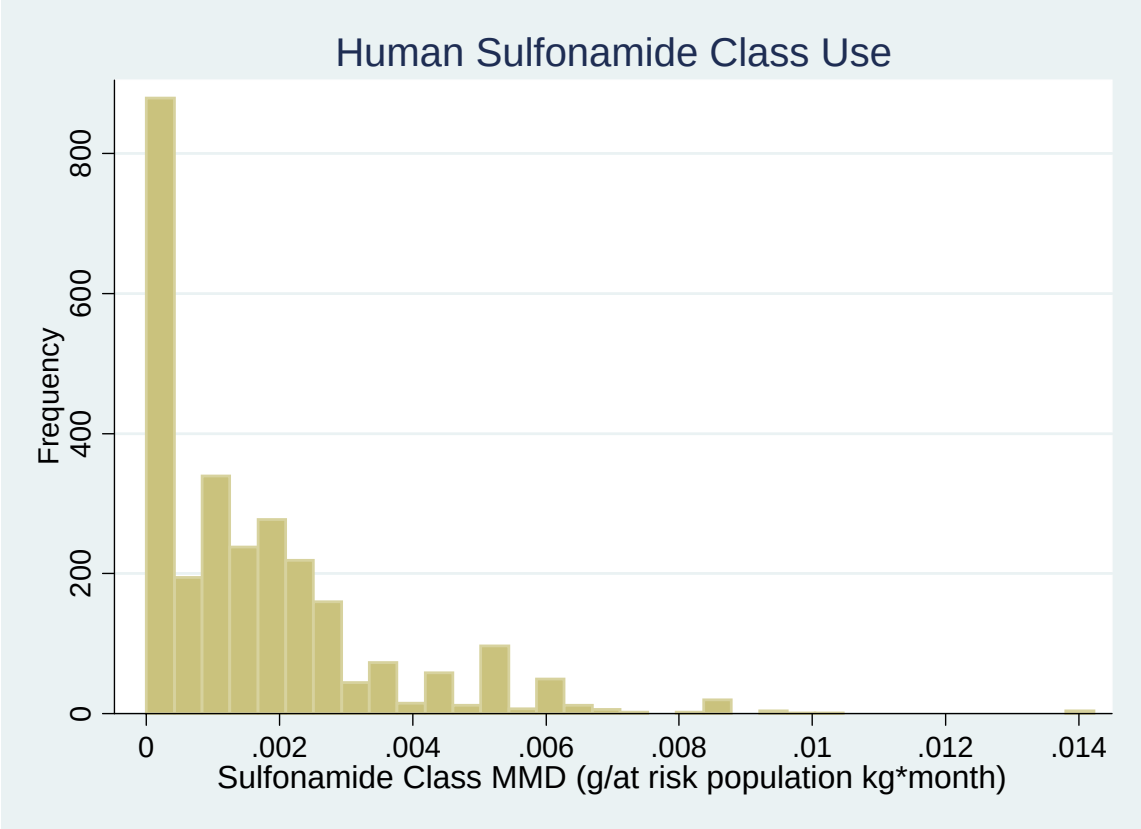

Figure 12. The human sulfonamide class use across all units from January 2004-January 2007.

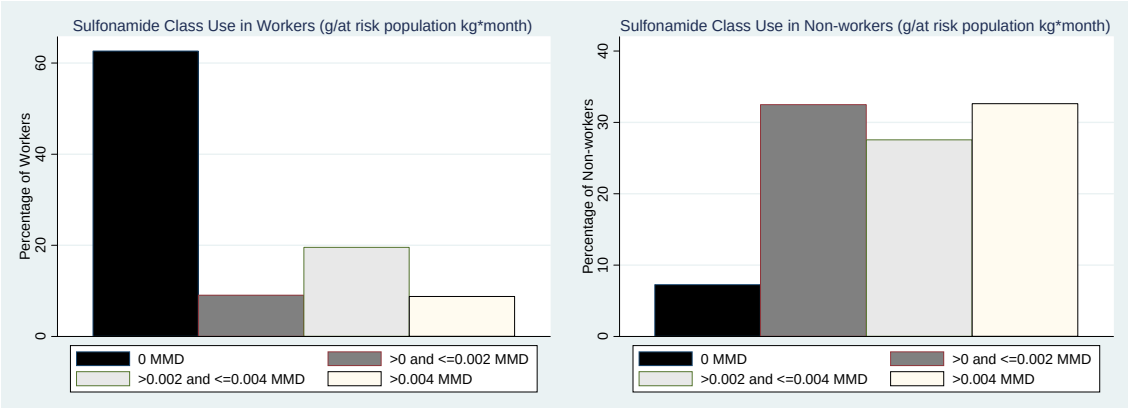

Figure 13. Sulfonamide class use distributed among the human categories.

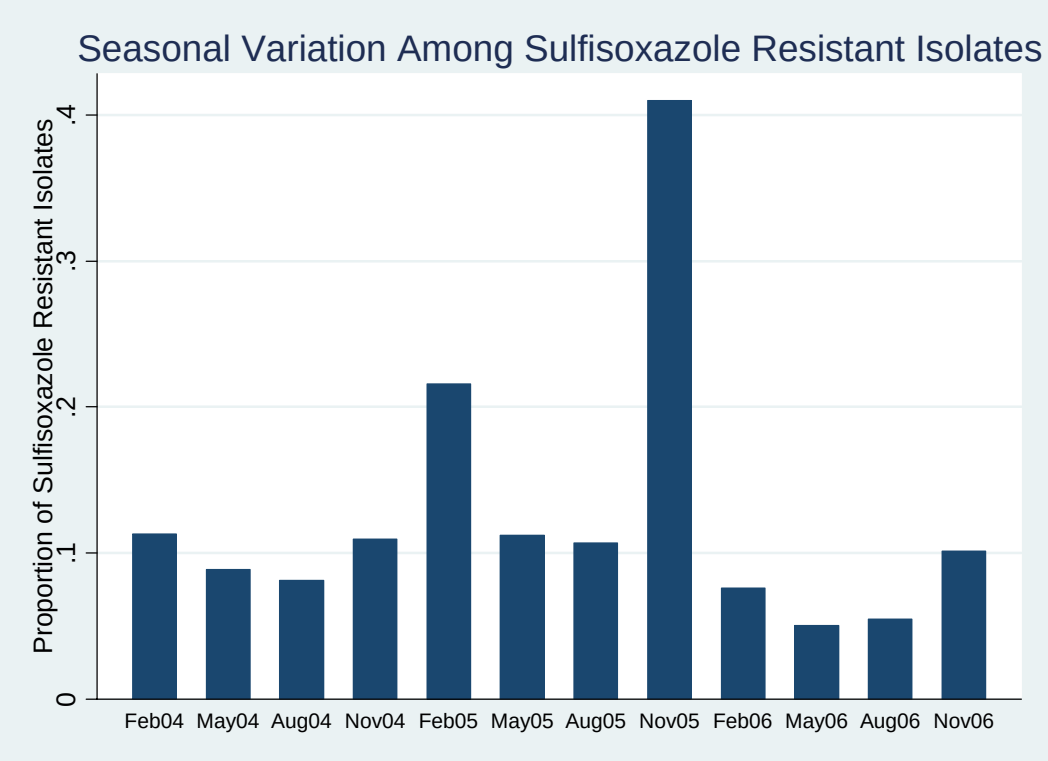

Figure 14. The proportion of sulfisoxazole resistant human *Escherichia coli* isolates sampled from February 2004 – November 2006.

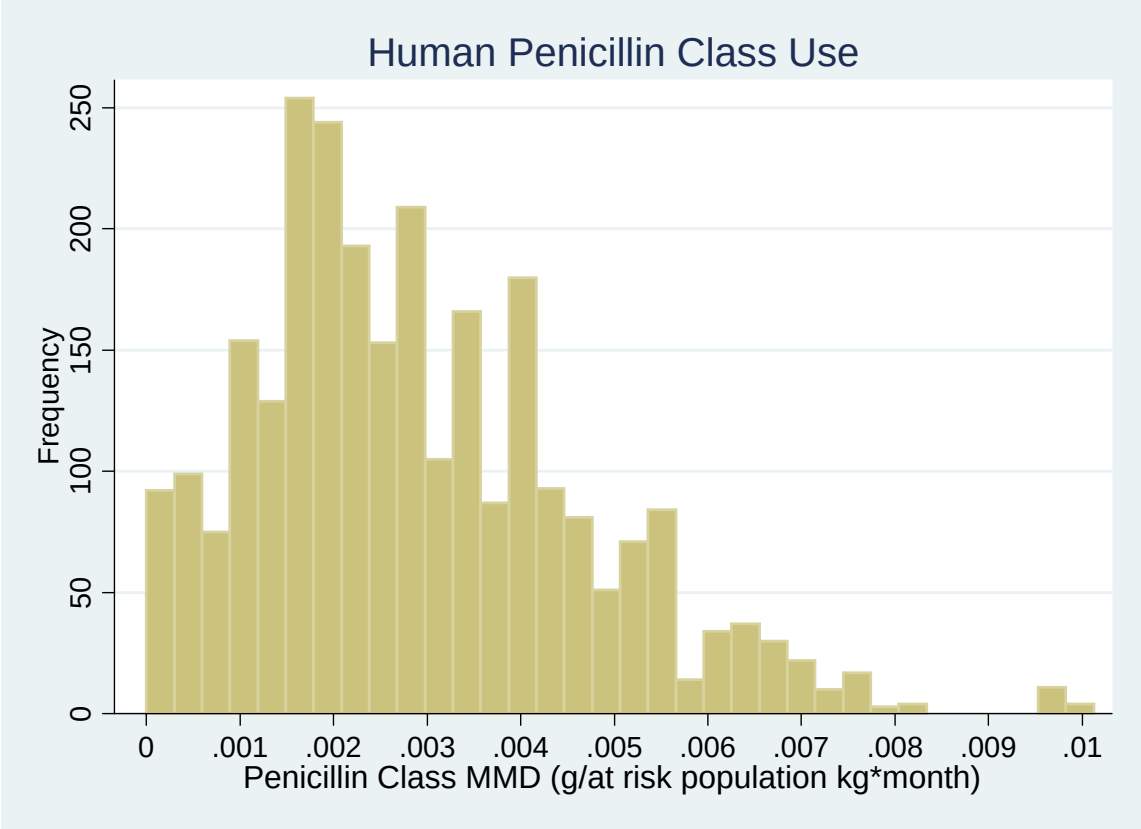

Figure 15. The human penicillin class use across all units during the period of January 2004-January 2007.

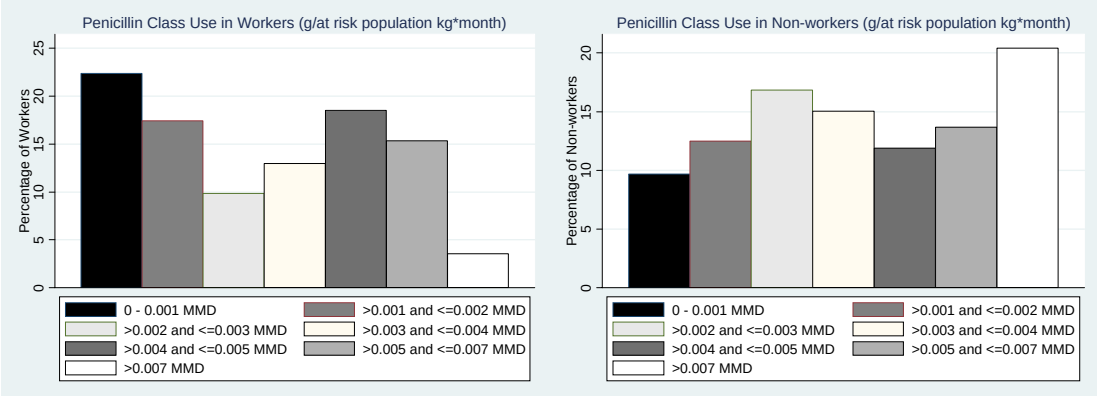

Figure 16. Penicillin class use distributed among human categories.

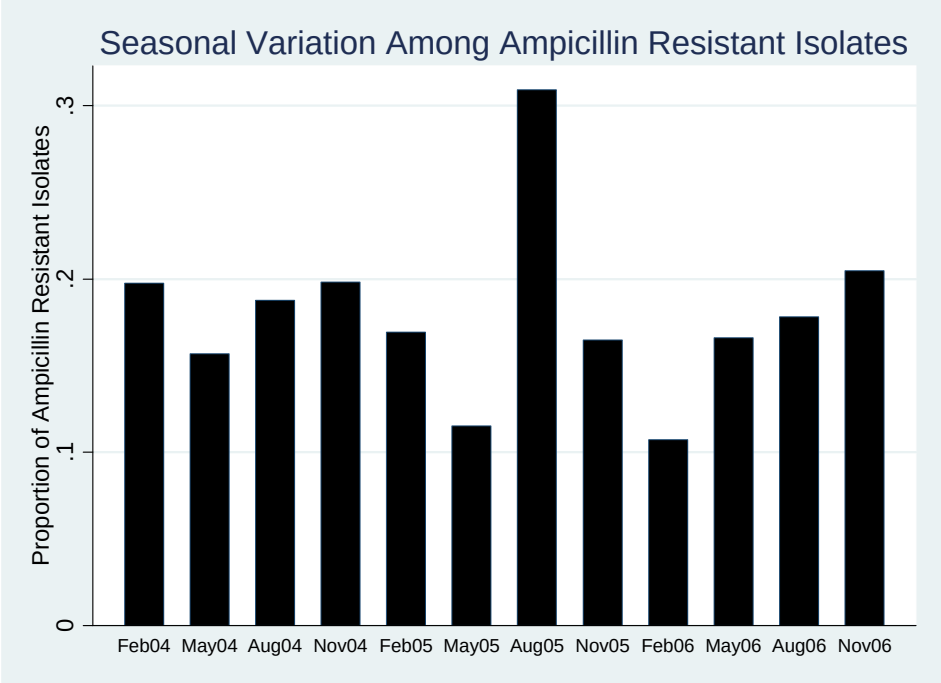

Figure 17. The proportion of ampicillin resistant human *Escherichia coli* isolates sampled from January 2004-January 2007.

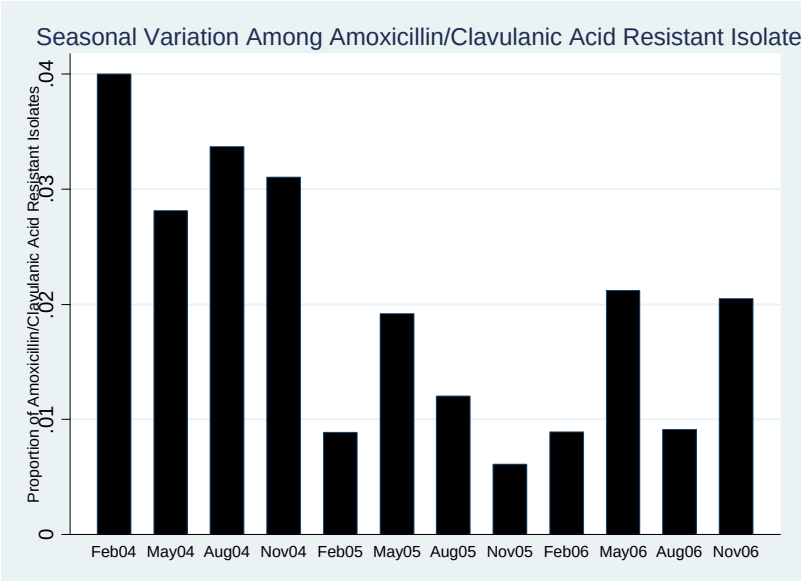

Figure 18. The proportion of amoxicillin/clavulanic acid resistant human *Escherichia. coli* isolates from February 2004-November 2006.

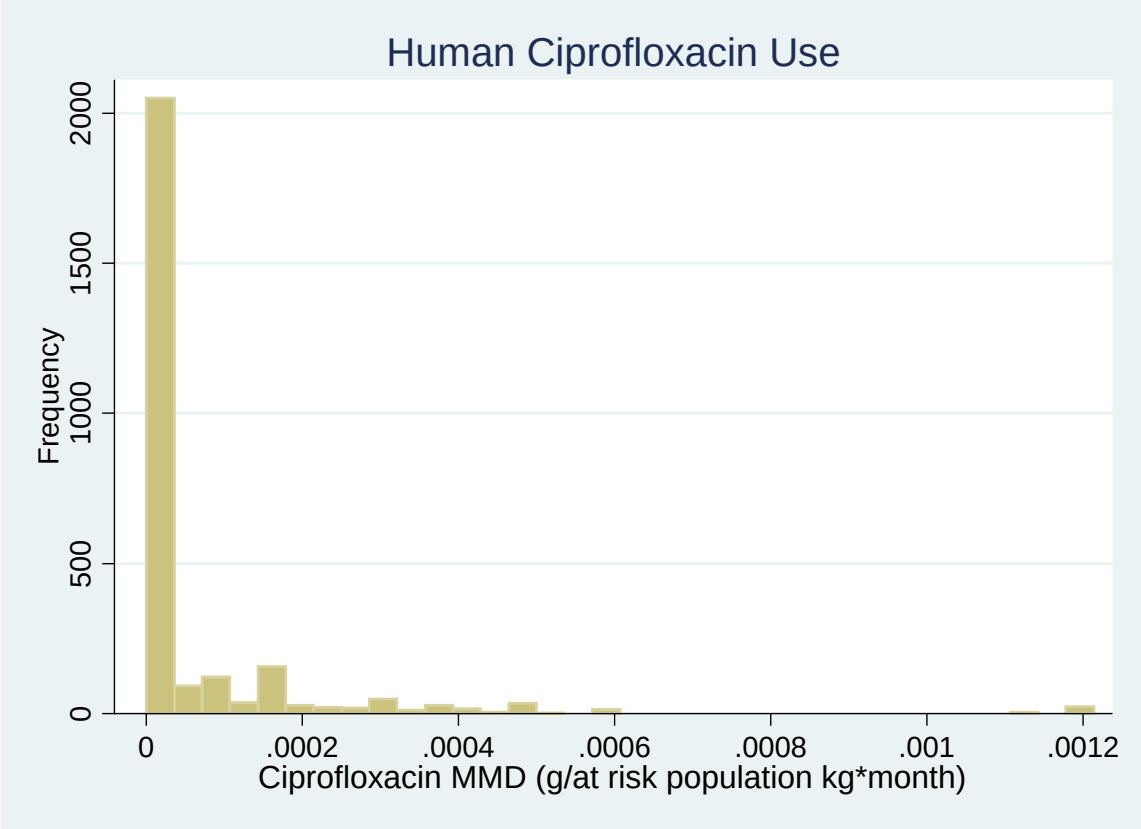

Figure 19. The human ciprofloxacin class mean monthly dosages across all units during the period of January 2004-January 2007.

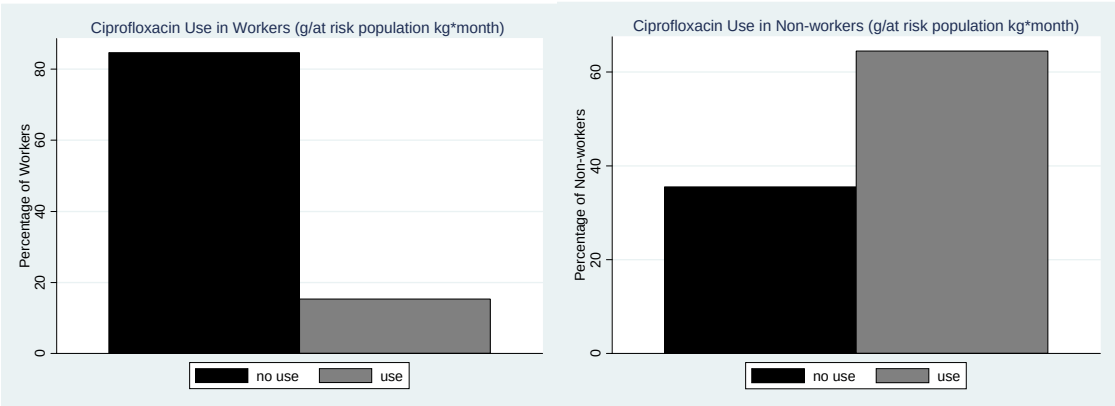

Figure 20. Ciprofloxacin use distributed among human categories.

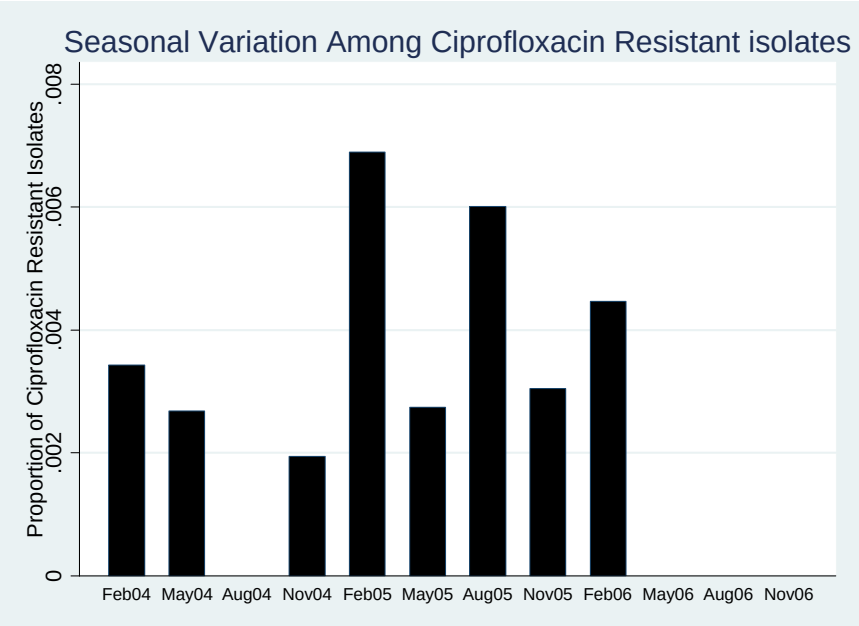

Figure 21. Proportion of ciprofloxacin resistant human *Escherichia coli* isolates from February 2004-November 2006.

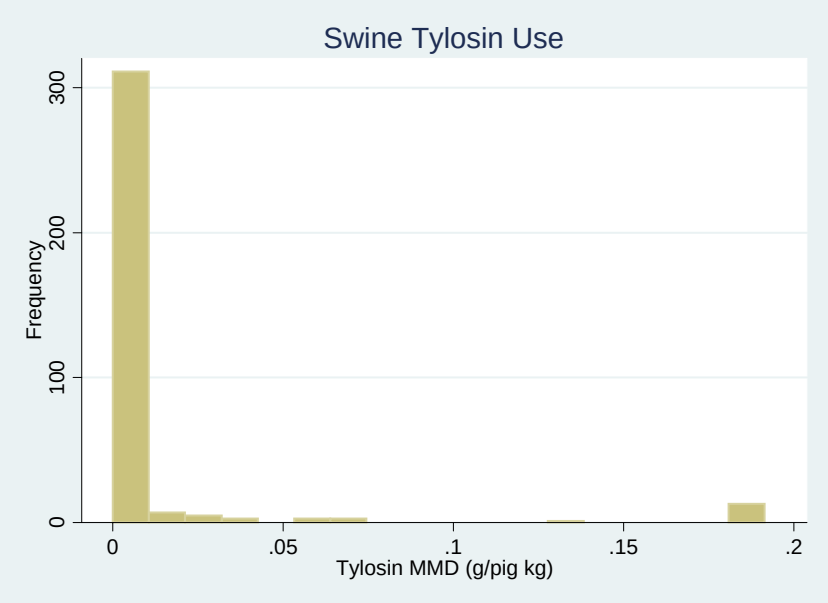

Figure 22. The swine tylosin mean monthly dosages across all units during the period of January 2004-December 2004.

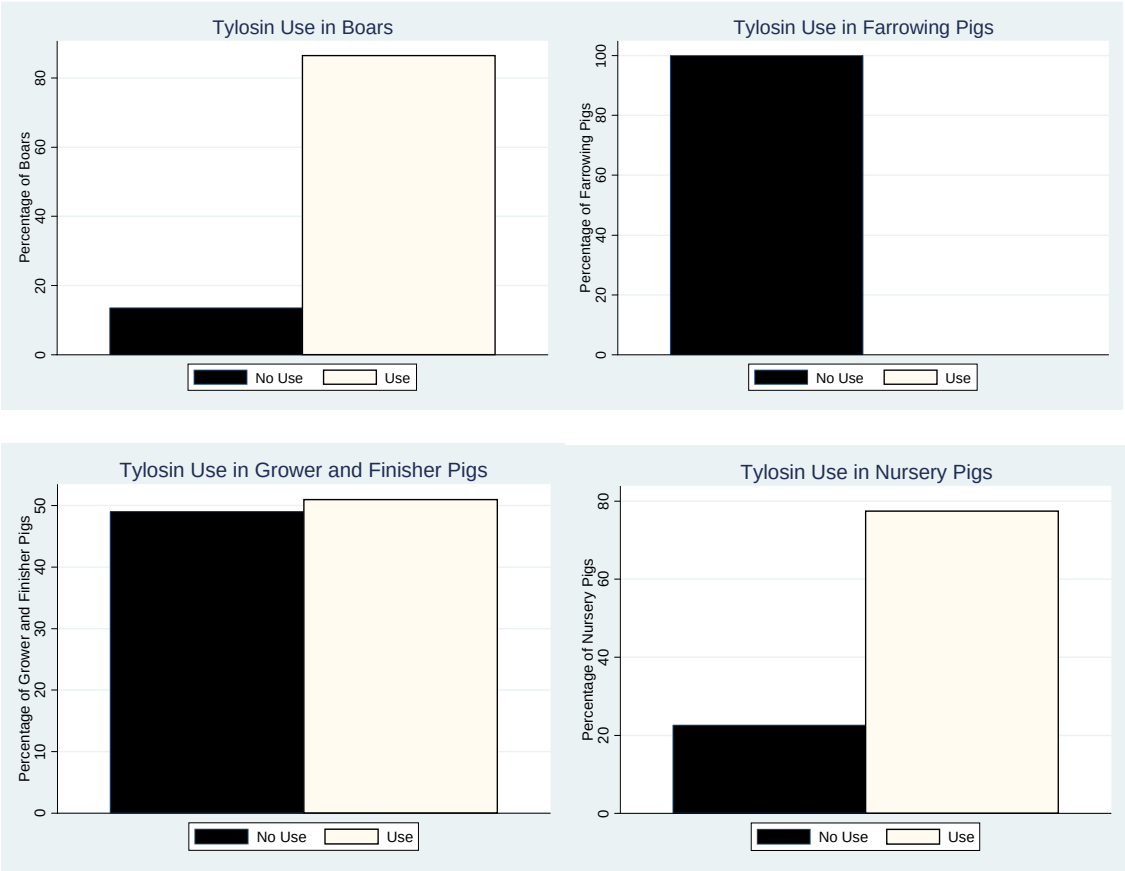

Figure 23. Tylosin use distributed across swine production groups.

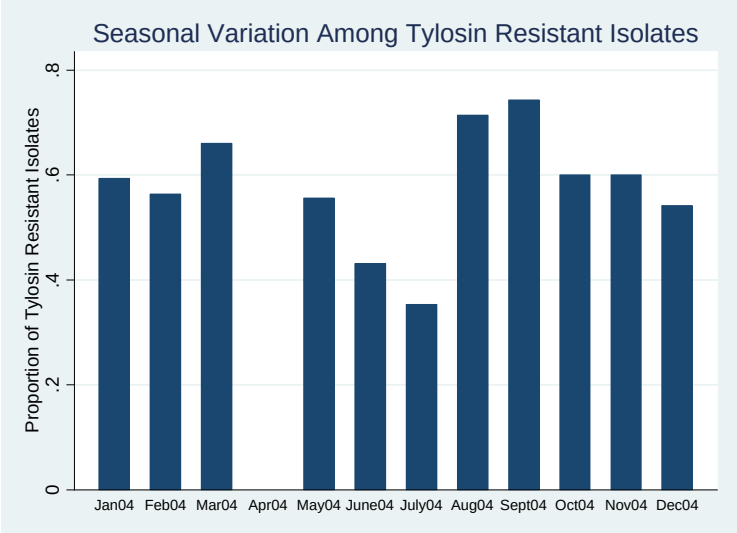

Figure 24. Proportion of tylosin resistant swine E*nterococcus* spp. isolates from January 2004-December 2004.

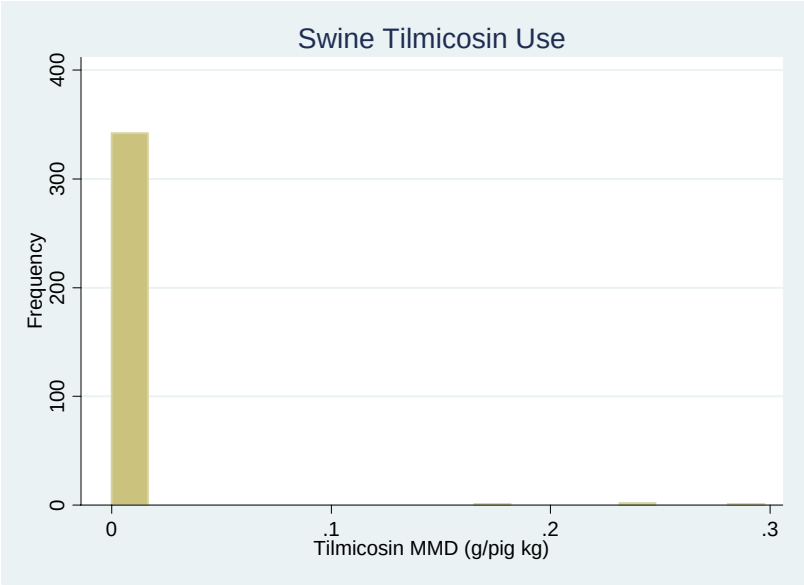

Figure 25. The swine tilmicosin mean monthly dosages across all units during the period of January 2004-December 2004.

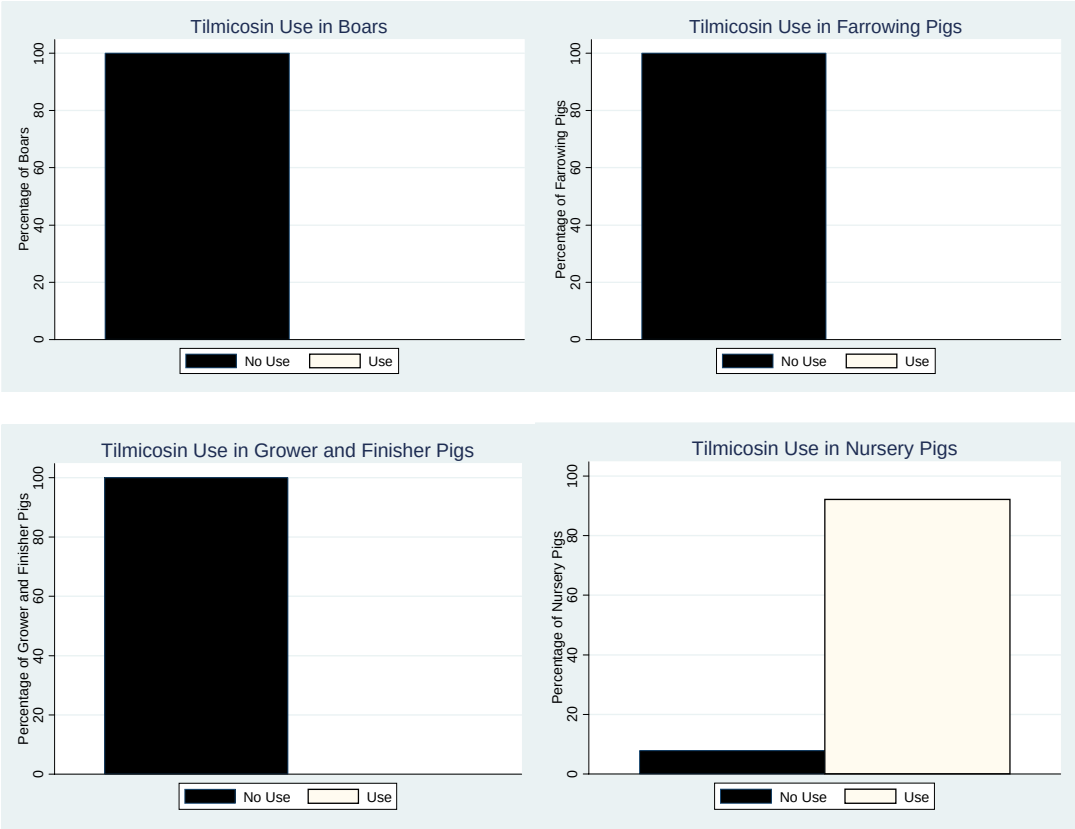

Figure 26. Tilmicosin use distributed among the swine production categories from January 2004-December 2004.

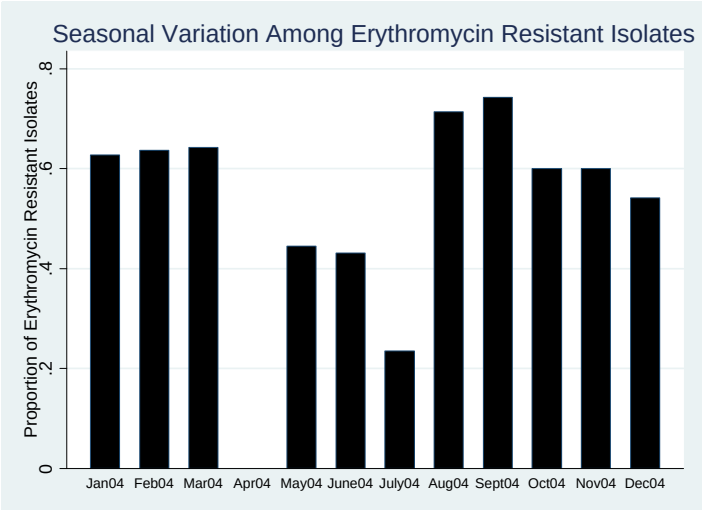

Figure 27. The proportion of erythromycin resistant swine *Enterococcus* spp. isolates sampled from January 2004-December 2004.

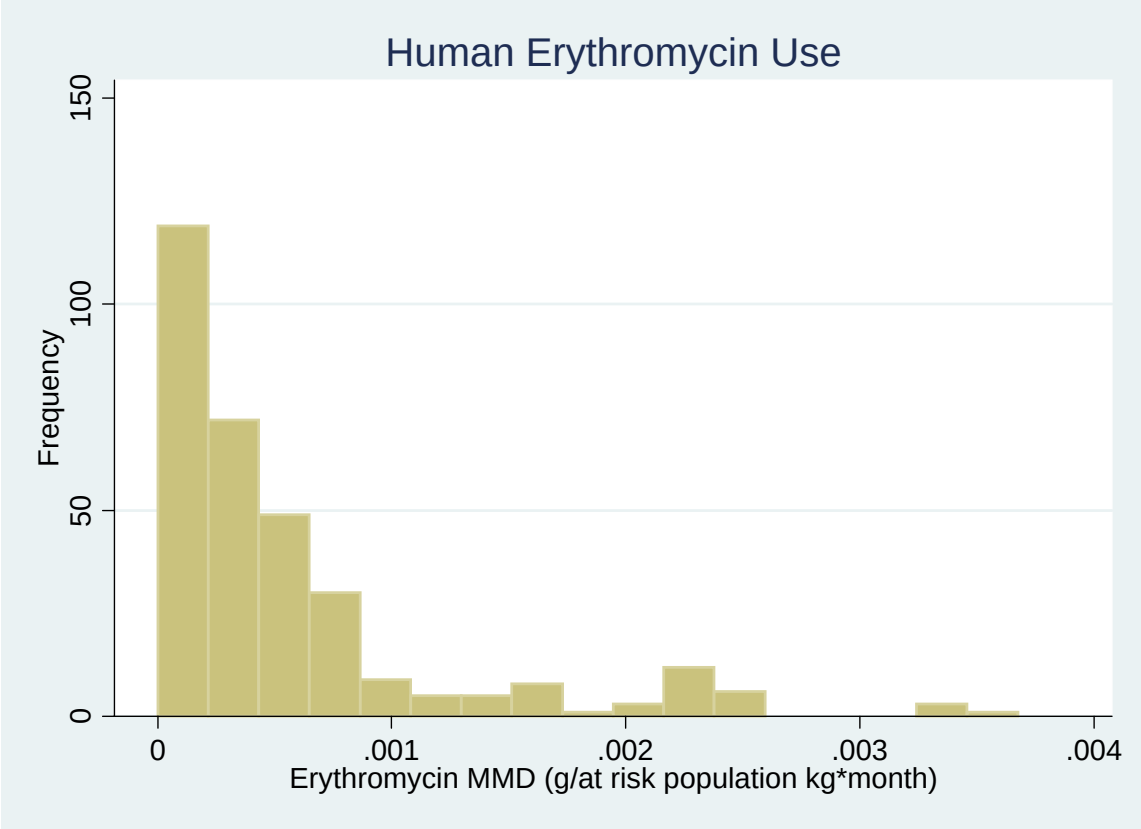

Figure 28. Erythromycin use across all human units from January 2004-December 2004.

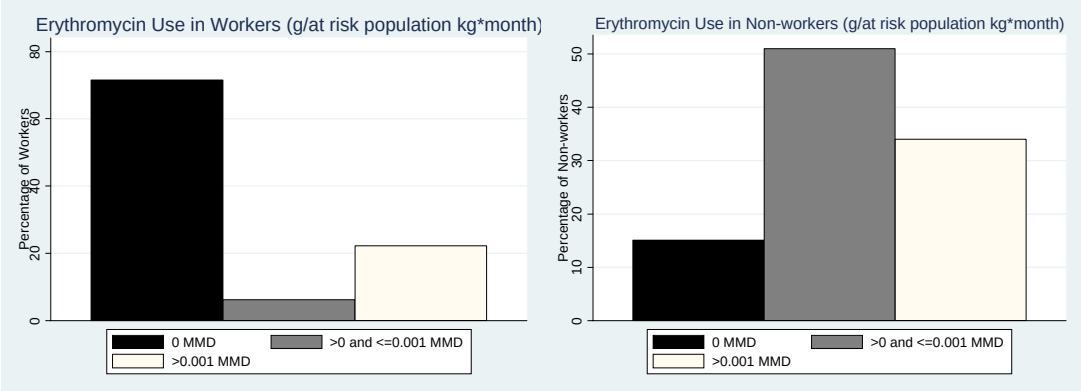

Figure 29. Erythromycin use distributed among the human categories.

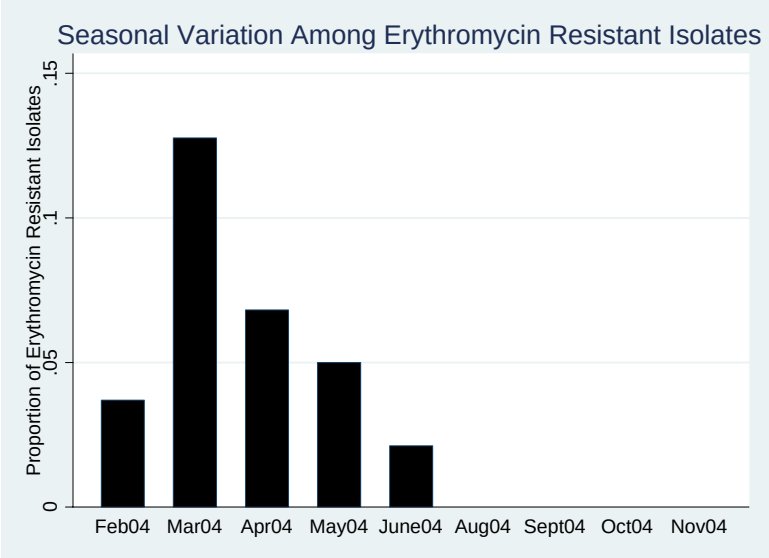

Figure 30. The proportion of erythromycin resistant human *Enterococcus* spp. isolates samples from January 2004-December 2004.

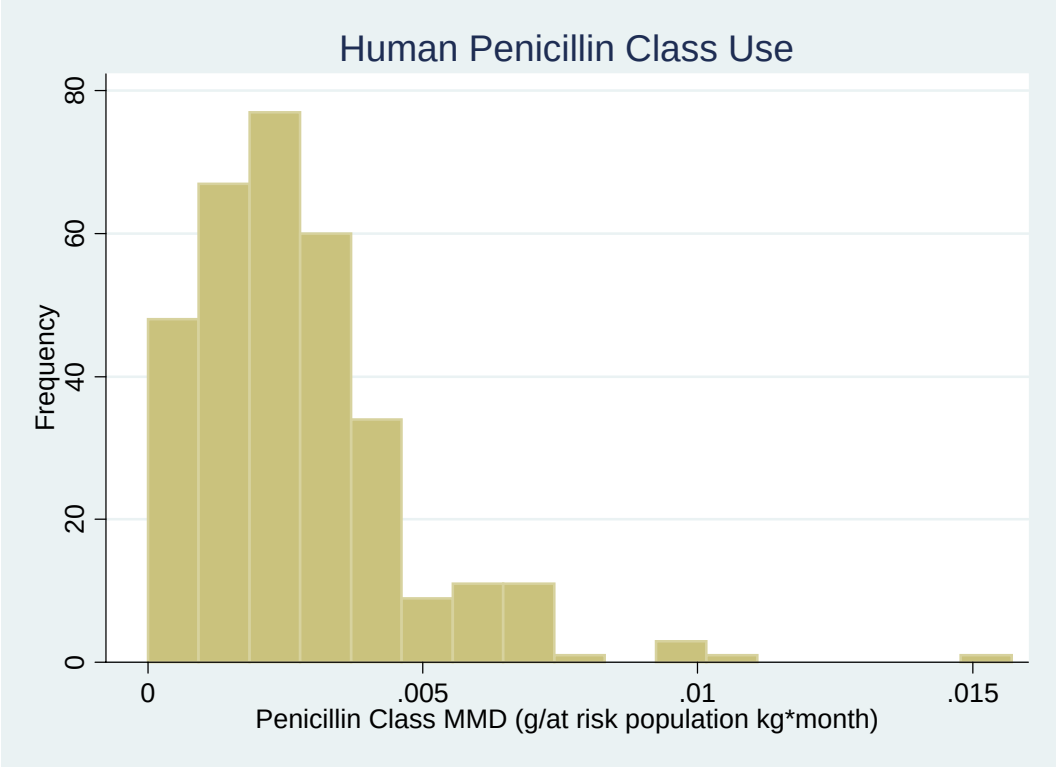

Figure 31. The penicillin class use across all human units from January 2004-December 2004.

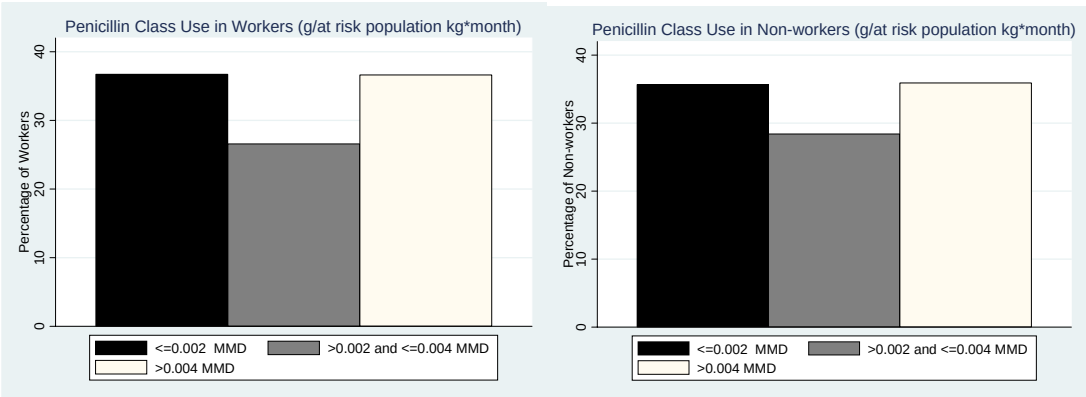

Figure 32. Penicillin class use distributed among the human categories.

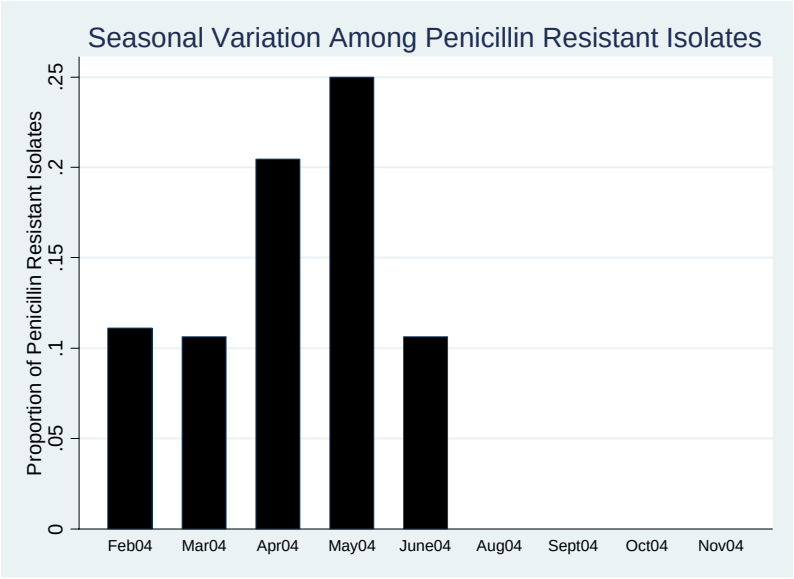

Figure 33. The proportion of penicillin resistant human *Enterococcus* spp. isolates sampled from January 2004-December 2004.

APPENDIX B

Table 1. Interpretation criteria of 15 antibiotics tested against *Escherichia coli.*

Antibiotic	**Range Tested**	**Resistant Breakpoint**
Amikacin	0.5 – 64	≥64
Ampicillin	1 – 32	≥32
Amoxicillin/Clavulanic Acid	1/0.5 – 32/16	≥32/16
Cefoxitin	0.5 – 32	≥32
Ceftiofur	0.12 – 8	≥8
Ceftriaxone	1 – 64	≥64
Chloramphenicol	2 – 32	≥32
Ciprofloxacin	0.015 – 4	≥4
Gentamicin	0.25 – 16	≥16
Kanamycin	8 – 64	≥64
Nalidixic Acid	0.5 – 32	≥32
Streptomycin	32 – 64	≥64
Sulfisoxazole	16 – 512	≥512
Tetracycline	4 – 32	≥32
Trimethoprim/Sulphamethoxazole	0.12 – 4	≥4

Table 2. Interpretation criteria of 17 antibiotics tested against *Enterococcus* spp.

Antibiotic	**Range Tested**	**Resistant Breakpoint**
Bacitracin	8 – 128	≥128
Chloramphenicol	2 – 32	≥32
Ciprofloxacin	0.5 – 8	≥8
Daptomycin	0.5 – 16	≥8
Erythromycin	0.12 – 4	≥4
Flavomycin	1 – 32	≥32
Gentamicin	128 – 1024	≥500
Kanamycin	128 – 1024	≥512
Lincomycin	1 – 32	≥32
Linezolid	0.5 – 8	≥8
Nitrofurantoin	2 – 64	≥128
Penicillin	0.5 – 16	≥16
Streptomycin	512 – 2048	≥1000
Quinupristin/ dalfopristin	1 – 32	≥4
Tetracycline	4 – 32	≥16
Tylosin tartrate	0.25 – 32	≥32
Vancomycin	0.5 – 32	≥32

Table 3. The 2004 *Escherichia coli* isolates grouped according to season.

Month	Months collapsed
February	February
	March
	April
May	May
	June
	July
August	August
	September
	October
November	November
	December
	January

Table 4. Swine *Escherichia coli* isolates collected from January 2004-January 2007 grouped by tetracycline resistance category and chlortetracycline mean monthly dosages.

Chlortetracycline MMD (g/pig kg)	Tetracycline Susceptible	Tetracycline Resistant	Total
0	316 (18.09 %)	1,431 (81.91 %)	1,747
>0 and ≤ 0.05	88 (16.18 %)	456 (83.82 %)	544
> 0.05	62 (6.72 %)	861 (93.28 %)	923

Table 5. The adjusted odds ratios for swine feces harboring tetracycline-resistant *E. coli* in relation to chlortetracycline mean monthly dosage.

Chlortetracycline MMD (g/pig kg)	Odds Ratio	Standard Error	z	P>|z|	95% Confidence Interval
0	1.0				
>0 and ≤ 0.05	1.20	0.186	1.18	0.239	0.885, 1.626
> 0.05	1.81	0.26	4.16	0.000	1.37, 2.40

Table 6. The adjusted odds ratios for harboring tetracycline-resistant *E. coli* in each swine production group.

Swine Production Groups	Odds Ratio	Standard Error	z	P>|z|	95% Confidence Interval
Farrowing	1.0				
Boars	1.51	0.255	2.47	0.014	1.09, 2.11
Grower & Finisher	1.24	0.175	1.58	0.115	0.948, 1.644
Nursery	1.38	0.277	1.61	0.106	0.933, 2.05

Table 7. Swine *Escherichia coli* isolates collected from January 2004-January 2007 grouped by ceftiofur use and susceptibility.

Ceftiofur Use (g/pig kg)	Ceftiofur Susceptible	Ceftiofur Resistant	Total
No	2876 (97.76%)	66 (2.24%)	2,942
Yes	261 (95.96%)	11 (4.04%)	272

Table 8. The adjusted odds ratios for harboring ceftiofur–resistant *E. coli* when ceftiofur was prescribed to the swine in the study.

Ceftiofur Use	Odds Ratio	Standard Error	z	P>|z|	95% Confidence Interval
No	1.0				
Yes	1.56	0.649	1.08	0.282	0.692, 3.53

Table 9. The adjusted odds ratios for harboring ceftiofur–resistant *E. coli* in each swine production category.

Ceftiofur Use	Odds Ratio	Standard Error	z	P>|z|	95% Confidence Interval
Farrowing	1.0				
Boars	0.179	0.115	-2.66	0.008	0.051, 0.636
Grower & Finisher	0.663	0.228	-1.20	0.231	0.033, 1.299
Nursery	2.61	0.931	2.70	0.007	1.303, 5.255

Table 10. Human *Escherichia coli* isolates collected from January 2004-January 2007 grouped by tetracycline class use and tetracycline resistance.

Tetracycline Class MMD (g/at risk population kg*month)	Tetracycline Susceptible	Tetracycline Resistant	Total
0 – 0.001	539 (76.56 %)	165 (23.44 %)	704
>0.001 and ≤ 0.002	676 (82.54 %)	143 (17.46 %)	819
> 0.002 and ≤ 0.004	431 (79.08 %)	114 (20.92 %)	545
> 0.004	151 (82.97 %)	31 (17.03 %)	182

Table 11. The adjusted odds ratios for harboring tetracycline–resistant *E. coli* in human study population in relation to tetracycline class use.

Tetracycline Class MMD (g/at risk population kg*month)	Odds Ratio	Standard Error	z	P>|z|	95% Confidence Interval
0 – 0.001	1.0				
>0.001 and ≤ 0.002	0.689	0.093	-2.76	0.006	0.529, 0.897
> 0.002 and ≤ 0.004	0.943	0.146	-0.38	0.706	0.696, 1.278
> 0.004	0.694	0.173	-1.46	0.144	0.426, 1.133

Table 12. The adjusted odds ratio for harboring tetracycline–resistant *E. coli* in the human population at risk category.

Tetracycline Class Resistance	Odds Ratio	Standard Error	z	P>|z|	95% Confidence Interval
Nonworker	1.0				
Worker	1.19	0.140	1.53	0.126	0.951, 1.51

Table 13. Human *Escherichia coli* isolates collected from January 2004-January 2007 grouped by sulfisoxazole resistance and sulfonamide class use.

Sulfonamide Class MMD (g/at risk population kg*month)	Sulfisoxazole Susceptible	Sulfisoxazole Resistant	Total
0	698 (85.64 %)	117 (14.36 %)	815
> 0 and ≤ 0.002	673 (84.87 %)	120 (15.13 %)	793
> 0.002 and ≤ 0.004	355 (84.73 %)	64 (15.27 %)	419
> 0.004	202 (90.58 %)	21 (9.42 %)	223

Table 14. The adjusted odds ratios for harboring sulfisoxazole–resistant *E. coli* in the human study population when an antibiotic belonging to the sulfonamide class is prescribed.

Sulfonamide Class MMD (g/at risk population kg*month)	Odds Ratio	Standard Error	z	P>|z|	95% Confidence Interval
0	1.0				
> 0 and ≤ 0.002	0.756	0.129	-1.63	0.102	0.541, 1.057
> 0.002 and ≤ 0.004	0.823	0.149	-1.07	0.285	0.576, 1.176
> 0.004	0.432	0.103	-3.53	0.000	0.271, 0.688

Table 15. The adjusted odds ratios for harboring sulfisoxazole–resistant *E. coli* in the human categories.

Sulfonamide Class Resistance	Odds Ratio	Standard Error	z	P>|z|	95% Confidence Interval
Worker	1.0				
Nonworker	1.67	0.265	3.27	0.001	1.229, 2.283

Table 16. Human *Escherichia coli* isolates collected from January 2004-January 2007 grouped by ampicillin resistance and penicillin class use.

Penicillin Class MMD (g/at risk population kg*month)	Ampicillin Susceptible	Ampicillin Resistant	Total
≤ 0.001	274 (86.71 %)	42 (13.29 %)	316
> 0.001 and ≤ 0.002	528 (82.50 %)	112 (17.50 %)	640
> 0.002 and ≤ 0.003	573 (88.29 %)	76 (11.71 %)	649
> 0.003 and ≤ 0.004	352 (83.22 %)	71 (16.78 %)	423
> 0.004 and ≤ 0.005	257 (84.26 %)	48 (15.74 %)	305
> 0.005 and ≤ 0.007	248 (76.54 %)	76 (23.46 %)	324
> 0.007	41 (83.67 %)	8 (16.33 %)	49

Table 17. The adjusted odds ratios for harboring ampicillin–resistant *E. coli* in relation to penicillin class antibiotic prescribing practices in the human study population.

Penicillin Class Use MMD (g/at risk population kg*month)	Odds Ratio	Standard Error	z	P>|z|	95% Confidence Interval
≤ 0.001	1.0				
> 0.001 and ≤ 0.002	1.43	0.325	1.58	0.115	0.916, 2.232
> 0.002 and ≤ 0.003	0.81	0.200	-0.84	0.403	0.502, 1.318
> 0.003 and ≤ 0.004	1.19	0.303	0.71	0.477	0.729, 1.965
> 0.004 and ≤ 0.005	1.25	0.327	0.86	0.388	0.751, 2.091
> 0.005 and ≤ 0.007	2.22	0.542	3.26	0.001	1.375, 3.583
> 0.007	1.39	0.704	0.65	0.515	0.515, 3.753

Table 18. The adjusted odds ratios for harboring ampicillin–resistant *E. coli* by population at risk category.

Ampicillin Resistance	Odds Ratio	Standard Error	z	P>|z|	95% Confidence Interval
Worker	1.0				
Nonworker	1.23	0.158	1.65	0.098	0.961, 1.589

Table 19. Human *Escherichia coli* isolates collected from January 2004-January 2007 grouped by amoxicillin resistance and penicillin class use.

Penicillin Class Use (g/at risk population kg*month)	Amoxicillin/Clavulanic Acid Susceptible	Amoxicillin/Clavulanic Acid Resistant	Total
≤ 0.001	310 (98.10 %)	6 (1.90 %)	316
> 0.001 and ≤ 0.002	624 (97.5 %)	16 (2.5 %)	640
> 0.002 and ≤ 0.003	637 (98.15 %)	12 (1.85 %)	649
> 0.003 and ≤ 0.004	411 (97.16 %)	12 (2.84 %)	423
> 0.004 and ≤ 0.005	298 (97.70 %)	7 (2.3 %)	305
> 0.005 and ≤ 0.007	316 (97.53 %)	8 (2.47 %)	324
> 0.007	48 (97.96 %)	1 (2.04 %)	49

Table 20. The adjusted odds ratios for harboring amoxicillin/clavulanic acid-resistant *E. coli* in relation to use of antibiotic belonging to the penicillin class.

Penicillin Class Use (g/at risk population kg*month)	Odds Ratio	Standard Error	z	P>|z|	95% Confidence Interval
≤ 0.001	1.0				
> 0.001 and ≤ 0.002	2.11	1.125	1.41	0.160	0.745, 5.99
> 0.002 and ≤ 0.003	1.015	0.594	0.03	0.980	0.323, 3.194
> 0.003 and ≤ 0.004	1.795	1.04	1.01	0.313	0.576, 5.591
> 0.004 and ≤ 0.005	1.679	1.01	0.86	0.389	0.516, 5.461
> 0.005 and ≤ 0.007	1.588	0.946	0.78	0.438	0.494, 5.106
> 0.007	1.984	2.204	0.62	0.537	0.225, 17.49

Table 21. The adjusted odds ratios for harboring amoxicillin/clavulanic acid-resistant *E. coli* in relation to population at risk category.

Penicillin Resistance	Odds Ratio	Standard Error	z	P>|z|	95% Confidence Interval
Worker	1.0				
Nonworker	1.17	0.343	0.54	0.592	0.659, 2.078

Table 22. Human *Escherichia coli* isolates collected from January 2004-January 2007 grouped by ciprofloxacin use and resistance.

Ciprofloxacin Use	Ciprofloxacin Susceptible	Ciprofloxacin Resistant	Total
No	1,901 (95.24 %)	10 (4.76 %)	1,911
Yes	796 (97.75 %)	7 (2.25 %)	803

Table 23. The adjusted odds ratios for harboring ciprofloxacin–resistant *E. coli* in the human isolates in relation to ciprofloxacin use within units.

Ciprofloxacin Use	Odds Ratio	Standard Error	z	P>|z|	95% Confidence Interval
No	1.0				
Yes	1.81	1.06	1.0	0.317	0.568, 5.737

Table 24. The adjusted odds ratios for harboring ciprofloxacin–resistant *E. coli* in relation to population at risk category.

Ciprofloxacin Resistance	Odds Ratio	Standard Error	z	P>|z|	95% Confidence Interval
Nonworker	1.0				
Worker	1.21	0.701	0.34	0.732	0.395, 3.76

Table 25. Swine *Enterococcus* spp. isolates collected from January 2004-December 2004 grouped by tylosin resistance and use.

Tylosin Use	Tylosin Susceptible	Tylosin Resistant	Total
No	144 (46.91 %)	163 (53.09%)	307
Yes	7 (17.95 %)	32 (82.05 %)	39

Table 26. The adjusted odds ratios for harboring tylosin–resistant enterococci in relation to swine tylosin use.

Tylosin Use	Odds Ratio	Standard Error	z	P>|z|	95% Confidence Interval
No	1.0				
Yes	3.54	1.656	2.70	0.007	1.415, 8.855

Table 27. The adjusted odds ratios for harboring tylosin-resistant enterococci in relation to swine production groups.

Tylosin Use	Odds Ratio	Standard Error	z	P>|z|	95% Confidence Interval
Farrowing	1.0				
Boars	2.13	1.158	1.39	0.164	0.735, 6.18
Grower & Finisher	0.451	0.112	-3.21	0.001	0.277, 0.733
Nursery	1.60	0.729	1.04	0.299	0.658, 3.908

Table 28. Swine *Enterococcus* spp. isolates collected from January 2004-December 2004 grouped by tilmicosin use and erythromycin resistance.

Tilmicosin Use	Erythromycin Susceptible	Erythromycin Resistant	Total
No	149 (43.57 %)	193 (56.43 %)	342
Yes	0	4	4

Table 29. The adjusted odds ratios for harboring erythromycin–resistant enterococci in relation to swine production groups.

Tilmicosin Use	Odds Ratio	Standard Error	z	P>|z|	95% Confidence Interval
Farrowing	1.0				
Boars	3.5	1.82	2.41	0.016	1.266, 9.686
Grower & Finisher	0.62	0.149	-1.99	0.047	0.388, 0.993
Nursery	1.91	0.857	1.45	0.147	0.796, 4.605

Table 30. Human *Enterococcus* spp. isolates collected from January 2004-December 2004 grouped by erythromycin use and erythromycin resistance.

Erythromycin MMD (g/at risk population kg*month)	Erythromycin Susceptible	Erythromycin Resistant	Total
0	59 (96.72%)	2 (3.28%)	61
> 0 and ≤ 0.001	204 (95.33 %)	10 (4.67 %)	214
> 0.001	47 (97.92 %)	1 (2.08 %)	48

Table 31. The adjusted odds ratios for harboring erythromycin-resistant enterococci in human isolates in relation to erythromycin use.

Erythromycin Resistance (g/at risk population kg*month)	Odds Ratio	Standard Error	z	P>|z|	95% Confidence Interval
0	1.0				
> 0 and ≤ 0.001	0.718	0.587	-0.40	0.685	0.145, 3.56
> 0.001	0.405	0.522	-0.70	0.483	0.0323, 5.071

Table 32. The adjusted odds ratios for harboring erythromycin–resistant enterococci in relation to population at risk category.

Erythromycin Resistance	Odds Ratio	Standard Error	z	P>|z|	95% Confidence Interval
Worker	1.0				
Nonworker	3.79	2.65	1.91	0.056	0.967, 14.89

Table 33. Human *Enterococcus* spp. isolates collected from January 2004-December 2004 grouped by penicillin class use and penicillin resistance.

Penicillin class MMD (g/at risk population kg*month)	Penicillin Susceptible	Penicillin Resistant	Total
≤ 0.002	122 (87.77 %)	17 (12.23 %)	139
> 0.002 and ≤ 0.004	124 (91.85 %)	11 (8.15 %)	135
> 0.004	47 (95.92 %)	2 (4.08 %)	49

Table 34. The adjusted odds ratios for harboring penicillin–resistant enterococci in human isolates in relation to use of antibiotics belonging to the penicillin class.

Penicillin Resistance (g/at risk population kg*month)	Odds Ratio	Standard Error	z	P>|z|	95% Confidence Interval
≤ 0.002	1.0				
> 0.002 and ≤ 0.004	0.654	0.281	-0.99	0.323	0.282, 1.518
> 0.004	0.334	0.257	-1.42	0.154	0.074, 1.509

Table 35. The adjusted odds ratios for harboring penicillin–resistant enterococci in human isolates in relation to population at risk category.

Penicillin Resistance	Odds Ratio	Standard Error	z	P>|z|	95% Confidence Interval
Worker	1.0				
Nonworker	1.33	0.532	0.71	0.479	0.606, 2.909

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